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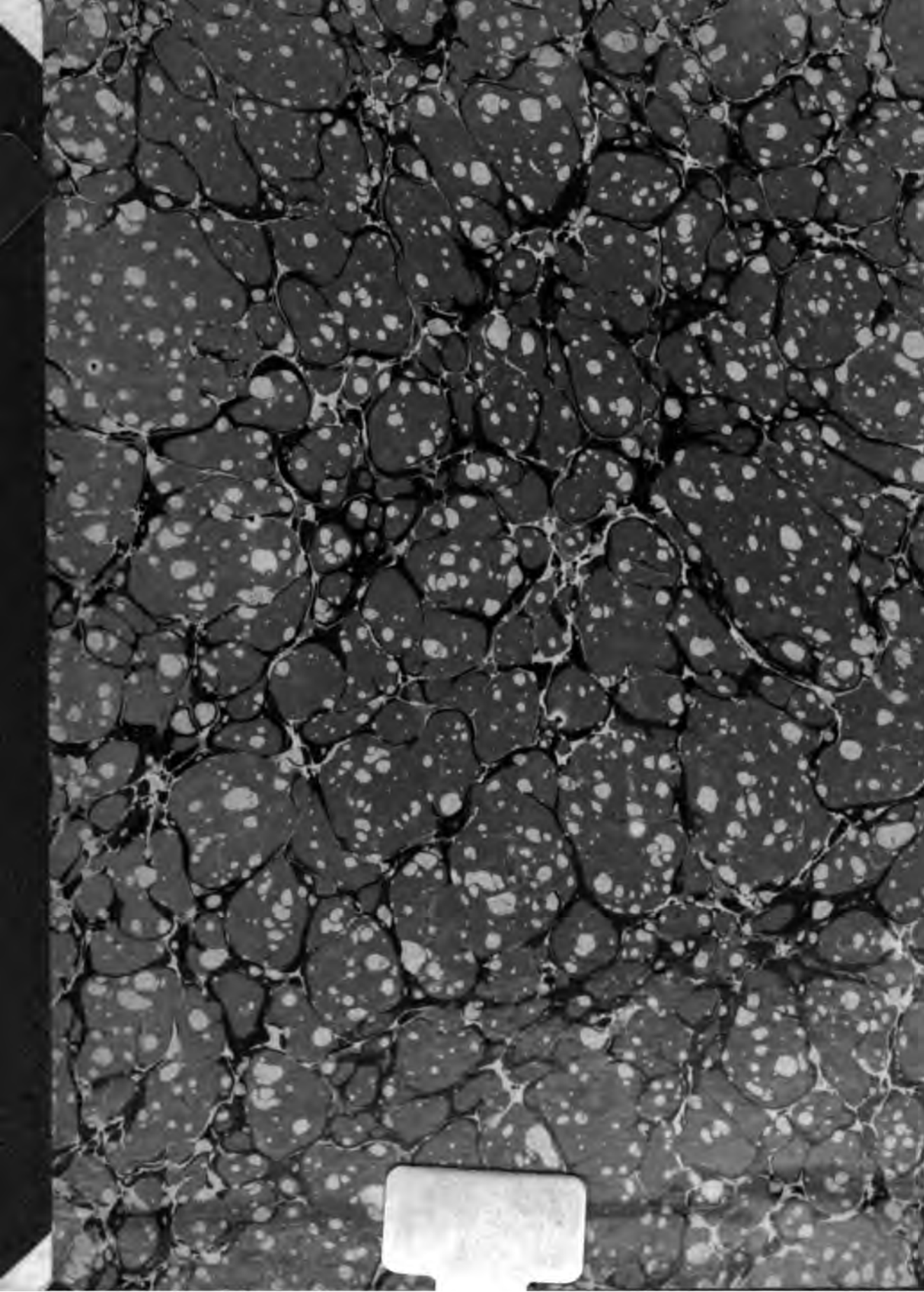
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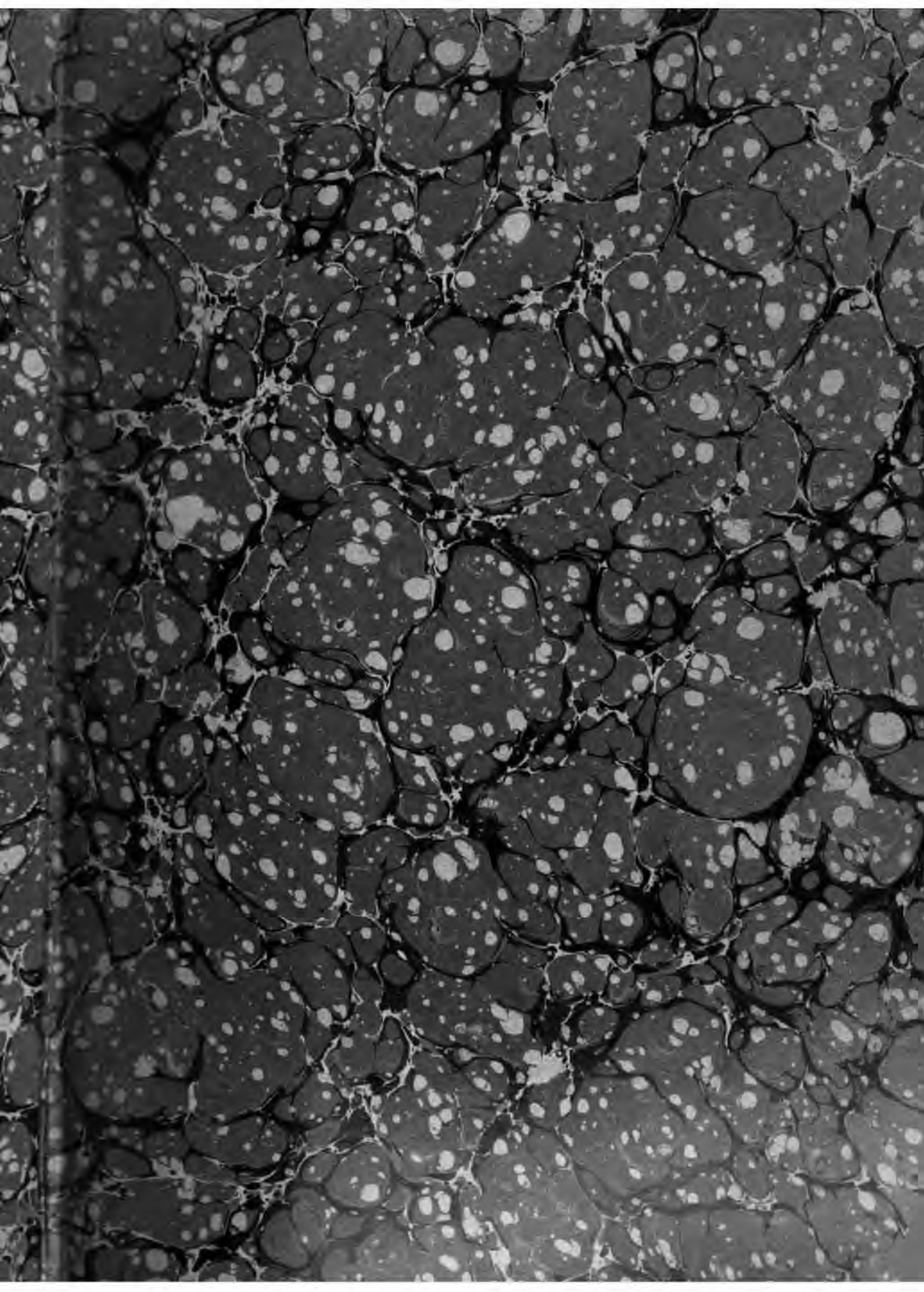
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BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

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BIOLOGICAL BULLETIN

THE OÖGENESIS AND EARLY DEVELOPMENT OF HYDRA.¹

RALPH E. WAGER.

During the winter of 1904-05 the writer took up the problem of the oögenesis of *Hydra*, especial attention being given to the cytological changes involved in the development of the egg. The species upon which the work was done was judged to be *H. grisea*. The animals were found growing in large numbers in a pond near Syracuse, N. Y. The results obtained were sufficiently marked to warrant publication, but the paper has been withheld until some of the points involved might receive confirmation from new material.

In the meantime there have been published two papers bearing upon the same subject : one by Tannreuther ('08) and another by Downing ('08). In general agreement with the results obtained by these two as well as by previous writers, were my own, but in some important particulars they differ widely. For several months past an abundance of sexually reproducing *Hydra* have been kept growing in the laboratory, and material taken therefrom has afforded ready confirmation of the results previously obtained. These results have been reviewed, and, considerably abbreviated, are here presented essentially unchanged. Some discussion of points suggested in recent papers has been inserted, and those particulars in which agreement with other workers exists have been but slightly dwelt upon.

As above stated, *Hydras* have been kept growing in the laboratory. Observations on these animals yielded interesting results. Early in October, 1907, collections were made from a small stream near Potsdam, N. Y., and the animals brought

¹ Contributions from the Zoölogical Laboratory, Syracuse University.

into the laboratory were placed in large gallon aquaria jars. In the jars were several species of unicellular, and some more highly organized plants in sufficient quantity to oxygenate the water. A few small pond snails were valuable in keeping the jars clean. In about two or three weeks after collection the animals began to bud very actively, and the dozen or so in each jar soon increased greatly in numbers. In one jar it was estimated that there were between two and three hundred individuals. This asexual period lasted between ten days and two weeks when there began an active sexual phase apparently as marked as had been the asexual. Throughout the winter months, and indeed, until observations were discontinued in July, 1908, this alternation of asexual and sexual periods was constant. At no time, however, were the reproductive activities entirely of one sort, but that there were periods of greatest sexual and of asexual development there could be no doubt. The same cultures have been kept under observation during the past year and somewhat similar though less marked conditions have been noted. The long vacation period during which they were without attention brought them to the fall of 1908 in a somewhat depleted condition. With fresh (distilled) water and food they began again to bud and produce eggs and spermaries. Their activities during the past few months, however, have been much less marked than formerly. This, I believe, to have been due in large part to the accumulation in the water of metabolic products deleterious to the normal activities of the animal. When taken from the jars and placed in fresh water with a plentiful supply of food, they began at once to bud rapidly and after a time to produce both eggs and spermaries, though, it must be said, with a vigor less than when the culture was first started. In proper environment, then, the *Hydra* will for a long time continue to reproduce both asexually and sexually, and the maximum development of each phase occurs in alternating cycles, or periods.

The embryos developed during the first sexual period were entirely of the flattened, short-spiculated form figured by Korotneff ('83) and Brauer ('91a) as that of *H. fusca*. These same animals, or at least their descendants, thereafter produced only the spherical heavily spiculate embryos figured by Kleinenberg

('72) as that of *H. aurantiaca*, and by Brauer (*op. cit.*) as belonging to *H. grisea*. It seems hardly possible that animals of both species (assuming *H. aurantiaca* and *H. grisea* to be identical) could have been taken under the same conditions and developed to such numbers at the same time. If but one species was present then it produced both forms of the embryo, while if both were present then one must have entirely disappeared and given place to the other. The latter alternative must have been attended by a marked diminution in the number of the animals immediately following the first sexual period, and no such observation was made. It appears then that the character of the embryo cannot be taken as a constant and specific difference. Brauer ('91a) uses this characteristic, together with the manner in which the embryo leaves the body of the mother, in separating *H. grisea* and *H. fusca*:

"*Hydra grisea* L. : Ei fällt ab, Form Kuglig, Schale ringsum mit grossen, an der Spitze meistverzweigten Zacken besetzt."

"*Hydra fusca* L. : Eier werden einzeln angeklebt, Form unten flach, oben konvex, Schale nur auf der oberen Seite mit Kurzen Stacheln besetzt."

An explanation of the cause for this unusual embryo-formation is not at hand. The conditions under which the spiculate embryos were produced were, so far as one could observe, the same as those under which the flattened, short-spiculate type were formed. It is rather remarkable that during the eighteen months and more that the animals were kept under observation, only the spherical, heavily spiculate embryos were to be found after the first sexual period during which the alternate type was produced. Moreover, the color of the animals under different conditions varied greatly, as did also their size and disposition toward sexual or non-sexual reproduction. Experiments were performed to determine, if possible, the relation of color, size, and reproductive proclivities to environmental factors. These were not altogether conclusive, but will be presented in another paper. In general, however, one may say that so widely do the animals vary under different conditions that one might easily mistake for a different species the forms encountered at either extremity of the varying limit. This, it seems, will readily account for the numerous

species described by the earlier investigators, who, naturally, placed much emphasis upon color and size as specific differences.

As killing agents, hot corrosive-acetic proved very satisfactory when the preparations were to be stained with Heidenhain's iron-hematoxylin. Corrosive sublimate, used both hot and cold, also gave good results. Bouin's mixture proved especially valuable in the study of the pseudo-cell formation.

ORIGIN OF THE OVARY.

Most investigators of the problem of the oögenesis of *Hydra* have attributed the origin of the ovary to the interstitial cells. My own observations have agreed in this respect. Longitudinal sections show that in the region in which the egg takes its origin there are numerous nests of these minute cells, but in the lower, or aboral end, they are much less numerous, and almost entirely absent near the foot. They are often found dividing mitotically, when there are usually three or four divisions taking place very near together. They vary somewhat in size in different sections, but their appearance and disposition would lead to the following conclusion with regard to their growth.

The very young interstitial cell is possessed of a nucleus which has a very delicate chromatin reticulum with small nodal enlargements. The nucleus, to a great degree, fills the whole cell, there being but a very thin envelop of cytoplasm. With the onset of egg production, the nucleus begins to enlarge and very soon a definite nucleolus can be found, and not infrequently, two are present. The mass of the cytoplasm increases at the same time and as a result of this increase in the size of these cells, a layer of the primitive ova, as they have been called, is definitely established. Externally, this is evidenced by the clitellum-like enlargement usually in the upper third of the body. This sometimes completely surrounds the body, or, with perhaps equal frequency, is found on one side only. Cases were noted in which no marked change in the external appearance could be seen, yet the process of egg production took place. The size of the egg is dependent in part upon the quantitative production of these cells, as will become evident from the discussion of their fate. Whether or not

the cells divide after reaching this stage, is rather uncertain. In none of the sections examined were there found conclusive evidences of such divisions taking place either mitotically or amitotically. There were present, however, many cases in which a cell was possessed of two nuclei. It was thought at first that division took place amitotically, for some of the nuclei were markedly elongated, and might easily give foundation for such belief. Such doubly nucleated cells probably arise from the coalescence of two cells.

The two nucleoli, above referred to as frequently being present in the nucleus, are not chemically the same, as they differ in their staining reaction, one staining much more deeply than the other (Fig. 1, Pl. I.). In some cases each of the nucleoli is found to be made up of two parts, in which again, one differs from the other in staining reaction. One appears to be a true nucleolus, while the other probably consists of a droplet of some food element passed through the nuclear membrane. Darkly staining bodies are also found in the meshwork of the cytoplasm (Fig. 1, Pl. I.). These appear to be true metaplasmic bodies, probably food material taken up by the cell. They are not found in all cases, and their presence is probably dependent upon the physiological activities of the cell. Frequently, the nucleolus migrates out of the nucleus by rupturing the nuclear membrane (Fig. 6, Pl. I.). The nucleolus is usually surrounded by a clear non-staining area. The chromatin material forms a fine network often somewhat peripherally arranged. In especially well-stained preparations one can distinguish very delicate threads which apparently hold the nucleolus in position. The nuclei themselves are either round or ellipsoidal in section. In the former case but one nucleolus is present, in the latter, two (Fig. 1, Pl. I.). Doflein ('97) describes similar conditions in the primitive ova of *Tubularia mesembryanthemum*, and Allen ('00) for *T. crocea*.

In the nests of interstitial cells from which the primitive ova are derived, one usually finds nematocysts developing in large numbers. In the course of development of the ovarian area these nematocysts either migrate out, or are resorbed. Frequently they are found within the egg itself.

GROWTH OF THE EGG.

The germinal area having been formed as above described, the egg begins its growth. Near the center of the mass of primitive ova there are to be found several which are larger than the rest. These cells lie close to the supporting membrane. Downing ('08) is inclined to regard these cells as the true ova, and is of the opinion that they are always present and recognizable among the interstitial cells of the adult animal. He notes that there are some cells which are spherical in outline, and are possessed of a very large nucleus near which there lies a small dark ovoid body. As noted above, the interstitial cells even in an early stage in the formation of the germinal area, differ considerably in size. The ovoid bodies near the nucleus are to be found in many of the interstitial cells lying near the supporting layer, and are doubtless only a metaplasmic body. It is permissible, certainly, to regard these cells as ova, and, as Downing contends, as true ova, but it would seem to be impossible to show that these large cells do not have precisely the same origin as the remaining ones of smaller size over which they have an advantage by virtue of their nearness to the entoderm from which their food supply is derived. All stages, as regards size, are to be found between these large and even the smallest cells. Unless these large cells do have a different origin than do the small ones, or unless they become markedly different in some respect from the small ones, there is nothing to be gained by regarding them as true egg cells and the small ones, therefore, as something entirely different. That these large cells may be present in the ectoderm is doubtless true. That the egg, in the manner to be described, takes its rise always from these and no other later formed large cells, seems to me to be extremely improbable.

The processes concerned with the growth of the egg are interesting. Tannreuther ('08) has already pointed out that the egg mass originates by the coalescence of a number of the primitive ova, the process beginning in the large cells near the center of the ovarian area. Inasmuch as my own observations were carried on in somewhat greater detail, it may be justifiable to outline the process again.

Reference has already been made to the large cells which,

in part, make up the primitive ova. In these cells begins the coalescing process, which is attended by marked cytological changes. The chromatin network retreats further from the nucleolus (Fig. 2, Pl. I., *a*), and finally assembles as a well-defined coarse spireme (Fig. 2, Pl. I., *b*). Coincidentally with this process, the nuclear membrane becomes less distinct and even absent in places. Within the nucleolus of these large, and frequently of even smaller cells, two or three minute vacuoles appear, and these may, in rare cases, number seven or eight. As the nucleus enlarges these vacuoles become larger and finally unite to form one which almost entirely fills the nucleolus; the chromatin filament becomes broken up into short strands; the nuclear membrane disappears entirely, and the nucleolus, frequently filled with the single large vacuole, very often migrates out of the chromatin strands (Fig. 5, Pl. I.). Associated with these processes is the disappearance of the cell-walls of those cells concerned in the changes. They become more and more indistinct and finally disappear entirely, or nearly so, as a result of which the substance of the several cells concerned unites to form a single mass (Fig. 4, Pl. I.). The mass formed by the fusion of these cells is the egg, in process of growth. It is interesting to note that there are several metaplasmic bodies in many of these cells as already described, but that now they are even larger and more pronounced.

The disappearance of the cell walls in the manner above described sets free the nucleus or its parts in the cytoplasmic mass of the egg. The coarse spireme of chromatin material becomes broken up into short strands which become dispersed and disappear entirely, though careful search in the cytoplasm of an egg will frequently reveal fragments whose presence therein is easily explained by the above account of the processes which take place (Fig. 9, Pl. II.).

Not all of the nuclei which contribute to the mass of the egg undergo this fragmenting process. Some persist apparently unchanged even for some time but their ultimate fate, save probably in the case of one, is in the formation of the so-called pseudo-cells which is to be described hereafter.

The egg mass now becomes distinctly vesicular, and imbedded

in it are a number of minute bodies apparently identical with those described as occurring in the primitive ova, and set free here by the coalescence of the cells. Some of them, however, are doubtless nucleoli, or at least nucleolar bodies, which, by the same process, have been added to the already rather complex egg mass.

The egg now continues to increase in size by the further appropriation of the cells surrounding it. The cells thus contributing their mass to that of the egg may undergo a regressive change similar to that described for the large ova which unite to form the growing egg. One of the primitive ova, or possibly at this time rightly called nourishing cells, becomes conspicuously larger than its fellows; its nucleus becomes correspondingly large and the regressive changes above described are repeated here. The chromatin assembles in a loosely coiled coarse spireme; the nuclear membrane disappears; the nucleolus becomes very large and frequently filled by a vacuole; the cell wall between this large cell and the egg gradually becomes absorbed and the two unite. Such a process in an intermediate stage is shown in Fig. 10 (Pl. II.) in which the nuclear membrane of the nourishing cell has entirely disappeared on one side and the chromatin filaments are broken up into short pieces. Two metaplasmic bodies are noticeable in the large cell as well as in the egg itself. Their probable origin has been discussed above. Fig. 9 (Pl. II.) represents a stage a little later than that above referred to. Outside of the egg is a large non-nucleated mass two or three times the size of the ordinary cells. Near it and next to the egg is a smaller cell in which these regressive changes are well advanced. The cell-walls between these two bodies is less distinct than in the other cells in the field, suggesting that the two would eventually unite, before union with the egg. Such conditions as this are not at all rare, and suggest that these large cells are developed by the union of two or more of the nourishing cells rather than by the growth of any one to this unusual size. Having reached this stage the large mass then becomes a part of the egg by the disappearance of the wall between the two bodies, thus setting free into the substance of the egg the chromatin strands, nucleoli and nucleolar bodies as

well as contributing considerably to the proportions of the egg by the addition of a considerable mass of cytoplasm.

It is evident from the above description that there is here an unusual case of nuclear degeneration. Processes of nuclear fragmentation have been described as occurring in the eggs of other coelenterates, but in a correspondingly later stage in their history and with ultimate results of an entirely different nature. Hickson ('93) has noted nuclear fragmentation in the case of *Distichopora violacea*; Allen ('00) in *Tubularia crocea*; Hargitt ('04) in *Eudendrium* and *Pennaria tiarella*. These cases are, however, quite different in that they consist of a simple breaking up of the nuclear elements into small parts without their entire dissolution. Herrick has described nuclear fragmentations in the Crustacea, viz., in *Alpheus minor*, *A. sauleyi* and *Homarus americanus* in which the nuclei constrict off small buds somewhat after the fashion of growing yeast. The process takes place for only a limited time in the egg-nauplius stage, and in definite regions. These cases differ widely from the one herein described in which the nucleus breaks down entirely and eventually disappears in the cytoplasm of the egg.

Soon after the egg has increased to a size six or eight times that of its original proportions, the nourishing cells are taken up in an entirely different fashion, and in a manner probably designed for supplying the embryo with energy in the later stages of its development. This process results in the formation of the so-called pseudo-cells. In some cases it does not begin until the egg is nearly one half its matured proportions. The beginning of the process is probably dependent upon the presence within the egg of some chemical compound which incites the changes to be described. The production of this substance is probably accomplished at different periods of development in different eggs.

Inasmuch as I have attempted to work out in some detail the cytological changes involved in the formation of the pseudo-cells, it may not be out of place to insert an outline of the opinions held by some previous investigators with reference to their origin.

HISTORICAL.

Kleinenberg ('72) first described the development of the pseudo-cells and proposed the name by which they have since been known. He remarked that they had been the source of many misconceptions, especially on the part of Ecker, who regarded them as true embryonal cells with a nucleus and a cell wall, and as arising by divisions of the original egg cell (*l. c.*, p. 39). He himself set about to find eggs in which all stages in their development could be found and came to the following conclusion with regard to their origin :

"Als Anfänge erscheinen sehr kleine kuglig umschriebene Verdichtungen im Plasma, die sich von den jungen noch nicht gefärbten Chlorophyllkörpern nur durch etwas stärkeres Lichtbrechungsvermögen unterscheiden. Indem sie sich vergrössern, entsteht in ihrem Innern eine Hohle die zuerst genau in der Mitte gelegen ist, mit dem fortschreitenden Wachstum aber diese centrale Lage verlässt und sich an einer Stelle der Oberfläche nähert. Hier erhebt sich darauf, frei in die Hohle hineinragend, auf breiter Basis ein niedriger kleiner Kegel, der später eine ungefähr linsenformige Gestalt annimmt und endlich zu dem Zapfen auswächst. Da sich die Eiweisskörner schon früher ausgeschieden haben, ist hiermit die Entwicklung der Pseudozellen beendet" (*l. c.*, p. 40).

This description is for the pseudo-cells of the egg of *H. viridis*. He states that a similar structure exists in those of *H. grisea* and *H. aurantiaca*, except that there is no plug or extension into the cell, but in its place a simple thickening of the rim of the cell which sometimes fills more than half its inner part.

Ciamician ('79) describes the formation of structures which he says "have the greatest similarity to the pseudo-cells found by Kleinenberg in the egg of *Hydra*." He, like Kleinenberg, believed that these bodies grow in the plasma of the egg for he states that "Die Pseudozellen erscheinen genau so wie bei *Hydra* anfangs als ganz kleine Kugelchen (0.001 mm. Durchmesser) und können bis zu einem Durchmesser von 0.005 mm. anwachsen." He describes also the vacuolated condition of some of these bodies and states that by the union of these vacuoles a certain nucleus-like part is cut out of the main mass. He found, too, that

these cells frequently divided into two, three, four or even six parts, usually all of unequal size. This process always took place before the cutting out of the central nuclear-like portion.

Brauer ('914, p. 170) makes mention of the "Dotterkernen, der sogenannten Pseudozellen." In describing the growth of the egg (p. 178) he speaks of "die Auflösung der Nahrzellen und ihre Aufnahme als sogenannte Pseudozellen durch die Eizellen." Further on in the same article (p. 184) he remarks that the structure of these bodies has been already described by Kleinenberg, implying that his own observations agreed with those of the last named author. In another paper of the same year ('916) he describes the origin of the pseudo-cells as follows: "Mit dem Wachsthum der Eizelle . . . beginnt die Auflösung der Nahrzellen, indem die Umrisse unregelmässig werden und der Kern verschwindet. Gleichzeitig treten auch die Pseudozellen als kleine, von Anfangs an sich intensiv farbende Kugelchen auf, welche rasch heranwachsen und die eigenthümliche Kernahnliche Form, wie Ciamician und Tichomiroff beschreiben, annehmen, sich aber von Kernen durch die starke Färbung besonders der peripheren Partie unterscheiden lassen." He then states that he agrees with Ciamician and Weisman as to their origin and that they do not arise directly from nuclei as Tichomiroff had shown, but that the latter author was deceived as to their true nature by their nuclear-like appearance.

Doflein ('97) describes in considerable detail the origin of the pseudo-cells. He cites the views of several of the authors named above, and then shows that in *Tubularia larynx* these structures are the metamorphosed nuclei of the cells which have fused with the egg and whose protoplasm has been added to it. These nuclei in the earliest stage of their metamorphosis differ from the nuclei of the primary germ cells only in that they lie in vacuoles. Their further metamorphosis consists in the disappearance of the vacuoles within the nucleolus, the thickening of the peripheral zone of chromatin matter, and the gradual disappearance of the clear non-staining area about the nucleolus. The nucleolus then enlarges considerably and the change is complete. By the use of a methyl-green-eosin stain he shows that a chemical change takes place in the nuclear substances during the meta-

morphosis. He observed that several of these persistent nuclei are often swept into the same vacuole by streaming movements of the protoplasm. He describes cases of the amitotic division of these nuclei as being of frequent occurrence within the egg.

Allen ('00) describes the changes undergone by the engulfed nuclei in the growing egg of *Tubularia crocea*. Many nuclei were found within the cytoplasm of the egg which showed no difference from the nuclei of the cells of the germinal layer.

These changes consisted in the assembling of the chromatin filaments into a varying number of small spheres just within the nuclear membrane, the disappearance of the threads supporting the nucleolus and a chemic change in the character of the ground substance supporting the chromatin, such that it becomes reactive to staining agents. The structure of the nucleus becomes less distinct. A description is also given of amitotic divisions of these nuclei within the cytoplasm of the egg. Her results agree largely with those of Doflein ('97).

Tannreuther ('08) observes that some of the nuclei of the interstitial cells surrounding the egg are taken up by it and transformed into pseudo-cells. The transformation is accomplished by the granulation of the chromatin; its arrangement in a band around the inner border of the nucleus, and the imbedding of the nucleoli within this band thus presenting the "appearance of a hollow sphere with its wall thickened on one side."

Downing ('08) believes them to consist of lecithin which at first is diffuse in the egg but later becomes aggregated to form the pseudo-cells. He notes also that the nuclei of the interstitial cells become filled with it, meantime enlarging considerably. These nuclei, he says, may also eventually give rise to the pseudo-cells.

It is evident from the above that two views have prevailed as to the origin of the pseudo-cell. The one, that held by the earlier writers principally, that they were accumulations or growths within the cytoplasm of the egg; the other, that they are the persistent and metamorphosed nuclei of the cells which fuse or coalesce with the egg in the process of its growth.

My studies have convinced me that they may be due to both of these processes, but that in the *Hydra* at least, these darkly

staining bodies within the cytoplasm of the egg are not alone nuclei, but also nucleoli, or whole cells, and in some cases, the equivalent of two or even three cells, in all of which a certain characteristic change has taken place.

An examination of Fig. 11, Pl. II., and Fig. 14, Pl. III., and Fig. 17, Pl. III., which are camera drawings of a portion of an egg in which these bodies are very darkly stained, will show that there are three or four different sizes; there is usually a darkly staining hemisphere while the other shades off almost imperceptibly into the surrounding cytoplasm, or at most has but a very thin rim of staining material; that some appear as a mere shell of stained matter with a non-staining interior; that a very few are exceedingly large when compared with the rest. All of these different kinds are not sharply differentiated but shade into each other as would naturally be expected from the consideration of the processes next to be described.

The account of the origin of these bodies, which are wrongly called cells, will be given under three headings: (1) Those derived from whole nourishing cells, (2) those derived from the nuclei of the nourishing cells, and (3) those derived from the nucleoli of the nourishing cells.

PSEUDO-CELLS DERIVED FROM WHOLE CELLS.

As previously indicated, the egg grows by two processes, one of which has already been described. The other is by the appropriation of the nourishing cells sometimes before any visible change either in size or appearance has taken place. This process is in some cases similar to those which take place when an amœba engulfs food particles. The cytoplasm of the egg gradually surrounds one of the cells and it is finally taken bodily into the egg. Cases of whole cells thus taken into the egg cytoplasm are not rare. Such a one is shown in Fig. 3, Pl. I. The cell wall is perfectly distinct and the cell contents differ little in appearance from those of one of the cells outside of the egg. The irregular, dark shading is a peripheral thickening of the protoplasm, as is evidenced by focusing upon different regions of the cell.

The fact that cells are frequently thus bodily engulfed lends considerable weight to the amœboid theory of the growth of the

egg. On the other hand, the process of adding cells in which an enlargement and regressive change has taken place has no similarity to amoebic activities. It appears, then, that the egg grows not only by processes comparable to amoebic activities, but also by those which have no possible similarity to them.

In all cases examined, the egg, just before breaking through the ectoderm, had appropriated all of the nourishing cells between it and the layer of large ectoderm cells (Fig. 14, Pl. III.). At either side of the egg mass a considerable amount of unused material may still remain after the egg has broken through the ectoderm but it is probable that it is used by another egg or, if conditions do not favor such a process, may be resorbed.

In many of the preparations the nuclei of the nourishing cells were possessed of several nucleolar bodies in addition to the nucleolus. These were sometimes darkly staining, but more often of a yellowish color. Often some were closely applied to the nucleolus and others to the nuclear membrane. Extremely irregular masses are often thus formed. Such nuclei are shown in Fig. 20, Pl. III. More will be said about them below.

When brought into the egg, and, as above noted, occasionally outside of it, a change is noticeable in the appearance and staining of the cells. In the case of whole cells these changes are as follows: The nucleus approaches the periphery of the cell; the nucleoli become peripherally arranged within the nuclear membrane and often appear to be pushing outward as the membrane is frequently extremely irregular and sometimes broken though this phenomenon is not always to be observed (Fig. 21, *a*, Pl. III.). The chromatin filaments are visible for a time but are much less distinct than in the normal nucleus. Whether or not the chromatin collects in the form of small spherules which resemble the nucleoli, I have been unable to determine. In some cases it seems to do so. Allen ('00) describes such a process for *Tubularia crocea*.

In the next stage the nucleolar bodies appear to coalesce in part, as their identity can no longer be recognized. The resulting mass is irregular in outline and very darkly staining. At the same time the region about it begins to react slightly to the stain (Fig. 21, *b*, Pl. III.). The chromatin filaments are now entirely

indistinguishable. The third stage resembles the one just described, but the hemisphere in which the nucleolar mass lies is also very darkly staining, so much so that the mass within it is often entirely obscured. By sufficiently destaining, however, one can distinguish this mass in almost any pseudo-cell of this sort. The nuclear membrane is broken early in the changes above described and is apparently entirely lost. The last stage is shown in Fig. 21, *c*, Pl. III. The drawings are taken from typical cases and many variations are to be found, but all are easily explained according to the above account.

Other pseudo-cells of much greater size are frequently found, and their origin can be explained in much the same way. Outside of the egg one sometimes finds that some of the cells have more than one nucleus, rarely as many as four or five. This fact has already been mentioned, and in connection with it a statement as to their probable origin, viz., by the union of two or more of the primitive ova rather than by the divisions of the nucleus of one of them. This conclusion was the result of the observation that no evidences of divisions in any of the nuclei of these cells were found, though such multi-nucleated cells were not uncommon. If divisions do occur the evidences are for their taking place amitotically, though I am inclined to believe that such divisions do not take place. The metamorphosis of these large cells into pseudo-cells takes place in the same way as that of the single-nucleated cell.

Occasionally a pseudo-cell of comparatively enormous size is found, such as that shown in Fig. 11, Pl. II. These are evidently derived from still larger multi-nucleated masses, which are sometimes found outside the egg. Fig. 19, Pl. II., shows such a cell with four nuclei and evidences of a fifth. I have found but this one instance, however, and it is therefore very unusual. Brauer ('91*a*) has shown a large pseudo-cell in his Fig. 4, Pl. X., and they are therefore not an unusual structure. The changes involved in their metamorphosis are probably the same as those already described. No intermediate stages were found and therefore no figures can be given.

In no cases have I found convincing evidences of the amitotic nuclear divisions of these bodies such as have been described by Ciamician ('79), Doflein ('97), Allen ('00) and Tannreuther ('08).

An explanation of the phenomena here described, though not easy to find, appears to be as follows : The nucleolar bodies when they appear within the nucleus in such large numbers are in small part products of the metabolic changes within the nucleus but in greater part are doubtless certain liquids which have osmotically penetrated the nuclear membrane and been coagulated by the killing and hardening fluids. These substances may serve as food for the nuclear activities and, in their later history, as stores of energy for the embryo. The ultimate disposition of these bodies in the cell would support this view. No attempt was made to determine their chemical nature by staining methods as the material required was lacking. Similar nucleolar bodies have been described by Montgomery ('98) in the case of certain nemertean worms.¹ By staining methods he seems to have shown quite conclusively that they are liquids taken in through the nuclear membrane and are of the same nature as the yolk balls which occur very abundantly within the cytoplasm. Such yolk masses are growths or accumulations and as such are different than the pseudo-cells which are found in the egg under discussion.

These nucleolar bodies usually appear in the first stage of the metamorphosis of the cell or nucleus. As indicated above, it is not probable that the chromatin collects in small spherules to add to the number. The nuclear membrane in this stage is frequently broken and very irregular. The second stage is due to a partial fusion or flowing together of these bodies. The rupture of the nuclear membrane results in the liberation of the nuclear liquids, in the case of the metamorphosis of a whole cell, which are apparently separated by some chemical factor so that the chromatin substance becomes spread out in the periphery of the hemisphere in which the nucleus lies, thus accounting for the darkly staining properties of this portion of the structure. At the same time the cytoplasm itself becomes chemically changed, for it reacts slightly to a chromatin stain. The nuclear sap does not appear to take any differential stain but is disseminated through the cytoplasm of the cell.

That this is the true explanation is evidenced by cross-sections

¹ Principally in *Stichostemma eilhardi*, p. 437; *Zygonemertes virescens*, p. 483; *Tetrastemma elegans*, p. 431.

of the pseudo-cell (Fig. 16, *a* and *b*, Pl. III.). Here one sees a thin rim of darkly staining material which is usually more prominent in one hemisphere though occasionally a continuous one is found (Fig. 16, *b*). Within this rim are the nucleolar bodies often forming a considerable mass or, as in the figure, spread out singly with their edges closely applied to each other. The interior of the structure takes a plasma stain but slightly and is very homogeneous in character. Fig. 16, *c*, Pl. III., is a drawing of a pseudo-cell in which some of the nucleolar bodies were in the pole opposite to that in which the darkly staining mass was found. They are evidently of a different nature than the others as the stain is apparently largely a plasma one.

The cause for the changes above outlined may possibly be due, as previously noted, to a substance secreted by the cytoplasm of the egg. This evidently causes a separation of the nuclear elements and they become arranged as above described. Further study on the processes which take place in the absorption of these bodies may serve to make clear the advantage, if any, which results from the arrangement of the material after the fashion above described. Sometimes the pseudo-cell lies in a conspicuous vacuole and at others none can be distinguished. The metamorphic changes are not entirely comparable to digestive processes for they cease for a time with the peripheral disposition of the darkly staining elements. The nuclein, which is highly resistant to digestive ferments, may serve to protect the contents of the structure from further changes.

2. PSEUDO-CELLS, DERIVED FROM NUCLEI.

There are a considerable number of pseudo-cells which are derived from the nuclei of the nourishing cells. In such cases, after being taken into or fusing with it, the cytoplasm seems to blend with that of the egg and then changes occur in the remaining nuclei which are similar to those described as taking place in the nucleus of the whole cells which are transformed into pseudo-cells. The nucleolar bodies do not cause the rupture of the nuclear membrane, but there is an evident separation of the nuclear elements, as the peripheral darkly staining rim clearly shows (Fig. 17, Pl. III.). This process is comparable

to that described by Doflein ('97) and Allen ('00) for the pseudo-cells of *Tubularia*. I have found no cases of division of these bodies.

3. PSEUDO-CELLS DERIVED FROM NUCLEOLI.

It has already been shown that the nucleolus of the nourishing cell sometimes migrates out of the nucleus, leaving a broken and partially destroyed nuclear membrane. After such a cell becomes a part of the egg the nucleolus may persist and form one of the minute bodies so plentiful in the egg. Fig. 6, Pl. I., shows the nucleolus migrating out of a nucleus before the union of the cell with the egg.

Nucleoli and other bodies within the nucleus are also set free in the egg cytoplasm by another process. Along the periphery of the egg one often finds examples of the apparent fusion of the cytoplasm of a cell with that of the egg followed by the enlargement of the nucleus in a manner slightly different from that described in the processes concerned with the transformation of whole cells into pseudo-cells. This condition was most common in those preparations which showed the food spherules in the nourishing cells. The outline of the nuclear membrane becomes irregular, often presenting the appearance of the nucleoli as being under an influence forcing them outward through the membrane. The outline of the nucleus may become twice as large as that of the normal. Fig. 20, *a* and *b*, Pl. III., are drawings of such nuclei but are by no means as irregular as many which were found. Whether these peculiar conditions ever result in the formation of a characteristic pseudo-cell or not, I have been unable to determine. That they do not seems highly probable. Their fate seems to be in the setting free of the nucleoli by the breaking or absorption of the nuclear membrane. Conditions which justify this conclusion are shown in Fig. 15, Pl. III., in which a nest of nucleoli are shown. Their appearance and arrangement indicate that the nuclear membrane had just disappeared as no trace of it could be seen. The minute bodies differed slightly in their staining reactions as indicated in the drawing. Probably, too, the metaplasmic bodies previously described as occurring in the cytoplasm of the egg persist and

swell the number of the minute spherules so plentiful in some preparations.

From the above accounts of the origin of the pseudo-cells it is easy to see how, ranging as they do from exceedingly small to very large ones, the conclusion might very naturally be reached that they are products of the cytoplasm of the egg and grow within it, undergoing certain changes in the process. Possibly the metaplastic bodies which have been described are derived from the cytoplasm. It is not improbable, then, that some of the darkly staining bodies, are, as Kleinenberg and others concluded, accumulations or growths within the egg, but the majority, so large that the process just mentioned may almost be disregarded, are the remains or parts of the nourishing cells which are taken into the egg, and, under certain influences undergo changes resulting in a partial breaking down, and a characteristic disposition of the elements composing them. These changes are not the same in all cœlenterate eggs in which pseudo-cells occur. The hydroid eggs which I have examined (*Tubularia*) showed no minute bodies at all. The pseudo-cells appeared to be derived entirely from the nuclei of the nourishing cells. Possibly the process here described may be found to occur only in periods of excessive sexual activity when food is very abundant.

NEMATOCYSTS WITHIN THE EGG.

In the early part of the paper it was noted that nematocysts may become imbedded in the egg. In several preparations, particularly in the region near the supporting layer, there were found oval bodies, apparently nematocysts, which were completely covered with minute spherical granules. In some few cases a suggestion of the filament within the nematocyst could be seen. The shape and position of these bodies led to the conclusion that they were nematocysts which were being absorbed or digested. The nature of the granules covering their surface is an open question. They may possibly have been digestive granules of some sort.

HISTORY OF THE NUCLEUS.

The early history of the nucleus is difficult to follow. As previously narrated, the egg begins its growth by the coalescence

of several cells whose cytoplasm unites to form a common mass and some of whose nuclei undergo certain degenerative changes while others apparently remain unchanged within the resulting multinucleate structure. It has also been suggested that all but one of these nuclei are transformed into pseudo-cells while that one becomes the functioning nucleus of the egg. In the early part of these studies it was thought that possibly all of the nuclei became functionless while the functioning nucleus was reformed *de novo*, from the nuclear material set free in the cytoplasm. The examination of a very large number of sections failed to furnish any growing egg without an apparently functioning nucleus. It seems therefore that from the beginning of the growth of the egg one nucleus retains its sovereignty, though it is impossible to maintain that for a time the others do not perform some of the nuclear functions. It is difficult, in examining the egg when in this multinucleate stage, to determine which is the functioning, or to be, the functioning nucleus. As soon, however, as it becomes distinctly recognizable, it has an appearance much as the one shown in Fig. 7, Pl. I. Both the nucleoplasm and the cytoplasm become more granular, but very shortly after, the nucleoplasm appears to be of an homogeneous character such as is shown in Fig. 8, Pl. I. At the same time the nucleoli increase rapidly in number, there being always one large one eccentrically placed within the nucleus. The staining reactions of the smaller nucleolar bodies indicate that they are not all of the same chemical composition. The nucleus is frequently found to be elliptic in section with its long axis at right angles to the supporting layer. The growth of the egg is attended by a corresponding increase in the size of the nucleus; the number of nucleoli increase greatly, while the nucleus, in the meantime, approaches the outer portion of the egg. In some cases there have been counted as many as eighty to ninety nucleolar bodies in a single nucleus. Vacuoles frequently appear in the larger ones.

The darkly staining area noted by Brauer ('91a) as occurring near the large nucleolus has been observed many times. It is shown in Fig. 9, Pl. II., and thus highly magnified, is, as he correctly observed, due to an aggregation of exceedingly small bodies, possibly of the same nature as the nucleoli.

There is a definite nuclear membrane closely applied to the cytoplasm.

In some cases a single cross-section has shown three eggs almost fully grown. Their separate masses in such cases are not separated by an egg membrane of any sort, but flow into each other at their boundaries, which are indicated by a lesser thickening of the egg mass. In such cases it appears that there are three distinct areas in which the growth of an egg has begun in the manner previously described. Tannreuther ('08) has made the observation that after the coalescence of the large ova, two or more of the nuclei persist and each becomes a functioning nucleus, and each, presumably, appropriating a portion of the original mass making up the cytoplasmic portion of the growing egg or eggs. My observations have given no evidences of such a condition or process, but indicate rather that egg development begins in two or more points or areas, and growth continues, until, by the total appropriation of the nourishing cells, their masses become continuous.

By the time the egg has attained its growth the nucleus has migrated to the periphery of the egg where it lies in an area free from pseudo-cells, and just beneath the ectoderm. The membrane becomes very indistinct, while a slight shrinkage away from the cytoplasm is noticeable (Fig. 14, Pl. II.). This condition becomes more pronounced for the outline of the nucleus becomes very irregular and almost indistinguishable. This condition, as Brauer ('91a) has stated, precedes the formation of the first maturation spindle.

MATURATION.

These processes have been described by Brauer ('91a) and Tannreuther ('08) for *Hydra* sp.?, and my own observations have added little to the account as given by them. The former writer states that the number of chromosomes is probably twelve or fourteen. Their shape and size make an accurate determination difficult, but I am inclined to consider that the number is as great as sixteen. The polar bodies are easily found in mature eggs, lying just beneath the ectoderm, and may remain attached to the egg by a cytoplasmic strand even after exposed to the water.

EXTRUSION OF THE EGG.

As previously noted, the egg begins by a process of coalescence, which may take place in more than one place, so that there may be three or four eggs growing on the same animal at the same time. In such cases the growing eggs form a thick band entirely surrounding the animal, and, in some cases, almost all of the upper third of the body. In other cases but one egg develops, when it appears as a well pronounced dome-shaped elevation, from which, before maturity, pseudopodial processes radiate outward, and may encircle over a half of the circumference of the body. In either case the completion of the process of appropriation of the nourishing cells is marked by the disappearance of the pseudopodial processes, and it then becomes more regular in outline, and more pronouncedly dome-shaped (Fig. 28, Pl. IV.). The ectoderm is then ruptured and quickly withdraws over the egg giving rise then to the bowl-shaped depression, or, if the extent of the area occupied by the egg or eggs makes this process impossible, the ectoderm is ruptured and the egg mass flows out through the opening gradually taking on the spherical shape and the shrunken ectoderm again forming the bowl-shaped depression in which the egg is found always to lie. The whole process rarely occupies more than two minutes and is sometimes accomplished even more quickly. The egg is attached to the body by delicate filaments continuous with the egg membrane.

FERTILIZATION.

The process of fertilization has been described by Brauer ('91a) and to his account little can be added. Unless the process is effected within a few hours after the extrusion of the egg, it becomes vacuolated and increases greatly in size, and finally goes to pieces. Numerous eggs were observed which, thus enlarged, gave off a part of themselves after the fashion of budding yeast. These parts thus set free were sometimes as much as one third the volume of the egg. Some eggs were found to be infested by a protozoön which fed upon the egg material. The interior of the egg in such cases contained hundreds of them. Apparently vigorous eggs were not found to be so attacked, but those in which degeneration was going on were frequently infested. It is

not impossible, however, that the healthy and vigorous egg may be sometimes thus infested.

EGG MEMBRANE.

For some time before breaking through the ectoderm the sections show an egg membrane of varying thickness to be present. This is sometimes difficult to find until shortly before the emergence of the egg. When the egg becomes surrounded by water the membrane is invisible owing to its transparency, but quickly becomes evident upon the application of killing fluids.

CLEAVAGE.

The first cleavage is total and equal. It begins at the distal pole of the egg and in a plane usually at right angles to the long axis of the body. Papillæ are well developed at the point where the cleavage begins and are easily seen on either side of the cleavage furrow when very young (Fig. 23, *a*, Pl. III.). This agrees with Kleinenberg's observations on the cleavage of *H. viridis*. The furrow deepens and its edges close over as it advances, its position being shown by an opening extending completely through the egg (Fig. 23, *b* and *c*, Pl. III.). The first cleavage is accomplished in about thirty minutes.

The second cleavage takes place in a plane at right angles to that of the first and is also total and equal. The papillary processes are also to be seen in the cleavage furrow. The four resulting blastomeres are of nearly equal size (Fig. 23, *d*, Pl. III.). The third cleavage takes place in a plane at right angles to the other two or parallel with the long axis of the body. In this case the cleavage furrow is much less distinct and is formed more slowly in some of the blastomeres than in others, with the result that from this point onward the blastomeres vary in size and give rise to an extremely irregular mass of cells (Fig. 23, *e*, *f*, *g* and *h*, Pl. III.). In the third and even later cleavages papillary prominences are frequently to be seen at the beginning of the cleavage furrow. These conditions greatly resemble those described by Kleinenberg for *H. viridis*. The blastomeres vary considerably in size, those at the proximal pole usually being of somewhat greater dimensions. At the conclusion of the third

cleavage the cleavage cavity is established (Fig. 24, Pl. IV.). After the third, the cleavages take place in such an irregular fashion as to make it impossible to follow them. As a result, however, of further divisions, there is formed a hollow mass of cells which in some cases vary but little in size, while other sections show a much greater variation (Figs. 26 and 27, Pl. IV.). This increases in size by the continued tangential division of the cells until a very large blastula is formed (Fig. 27, Pl. IV.). The inner ends of the cells contain the larger portion of the pseudo-cells while the nuclei are located near the outer part.

EMBRYONIC LAYERS.

The cells of the blastula above described are nearly of the same size, though some project into the cleavage cavity farther than others. Division of these longer cells occurs and the entoderm is thus formed by a process of delamination. With these daughter cells are carried the larger part of the pseudo-cells, so that when the process of entoderm formation is finished the ectoderm cells are comparatively free from them. By the continued division of the primitive ectodermal cells in this fashion the cleavage cavity becomes almost entirely obliterated. The entoderm cells also divide and the solid mass of cells is formed. That the ectoderm cells may themselves be crowded into the cleavage cavity as observed by Brauer ('91a) found no confirmation in the preparations examined. Radial divisions were the most numerous with few tangential divisions.

No uniformity in the size of the eggs produced was observed for the amount of material available for the growth of the egg determines in large part its ultimate size.

The completion of the formation of the entoderm marks the beginning of the processes resulting in the embryonic membranes, which, as in other species of *Hydra*, consist of an outer chitinous and densely spiculate shell and a very thin inner membrane just beneath it. The former takes its origin as follows: The ectodermal cells constituting the outer layer of the embryo send out slender processes as shown in Fig. 13, Pl. II., and Fig. 29, Pl. IV. These are most frequently formed in groups of two or more and are remarkably large when compared with the size of the

whole cell. Rarely more than one is produced by one cell so that the several processes constituting a group are formed from contiguous cells. The ends of the process show a marked curve away from the axis of the group. The growth outward of these processes carries with them at their extremities the egg membrane which remains a noticeable part of the embryo even at the time it is set free from the mother. The ectoderm cells at this time are very long and appear greatly distorted as a result of the great change in form. The outer part of the ectoderm cells then gives rise to a thin chitinous envelope as shown in Fig. 12, Pl. II. The cytoplasm within the envelope and near the extremities of the projections becomes very much more granular than the remaining portion, and takes a darker stain as a result. The chitinous covering continues to thicken, and the protoplasmic contents of the processes apparently give rise to the chitinous material of which the spicules are composed, whether by a transformation of the protoplasm into the chitin or by a process of secretion of the material of which it is composed, I am unable to state. The appearance of the protoplasmic granules just within the chitinous covering of the processes would indicate a deposition of material at that point. As this chitinous envelope becomes thicker, and of course harder, it gives rise to a very effective protective covering. From the above account it is evident that the covering takes its origin from the outer part of the ectoderm cells; the basal portion containing the nuclei and a few pseudo-cells remains apparently unchanged. Beneath the shell there is now a layer of cells of somewhat smaller dimensions but otherwise differing little from their condition before the formation of chitin. From the outer part of these cells there is then secreted the thin inner membrane, and the process is thus completed.

This account agrees in general with that given by Brauer (91a) for *Hydra* sp. ?. After the completion of the process of shell formation the embryo remains attached to the mother by means of two or three tough strands which are also somewhat chitinous. They are apparently identical with those holding the egg after its emergence from the ectoderm. In the meantime the shell becomes harder and sections show its characteristic laminate structure, that is, the delicate lines which give it an appearance

of being so constituted. Usually within two days the embryo falls from the parent and its point of attachment is then marked only by the bowl-shaped portion of the shrunken ectoderm by which the egg was originally covered.

No study was made of the processes concerned with the further development of the embryo and of the cytological changes involved in the transformation of the embryonic layers into the definitive ectoderm and entoderm, nor of the further changes in the pseudo-cells. That marked changes must take place becomes evident when one considers that in the ectoderm there must be developed muscle and nerve cells, interstitial cells and nematocysts, while the pseudo-cells are to be absorbed and utilized by the entoderm cells. It was found that embryos would develop and give rise to young *Hydra* in about three weeks. This, of course, was at the temperature of the laboratory. At the time of first appearance from the shell they are possessed of four short tentacles, which later increase to six or more. All traces of the pseudo-cells have disappeared by the time of the completion of the definitive ectoderm and entoderm.

GENERAL DISCUSSION.

As to the true nature of the growth of coelenterate eggs, two views have long been prevalent. One, commonly known as the amœboid theory, is so denominated on account of the striking likeness of the growing egg to an amœba — this likeness lying in the amœboid-like movements in its migrations from one tissue into another ; in that it often possesses pseudopodial processes like those of an amœba ; and more remarkable still, frequently engulfs neighboring cells much after the fashion of an amœba taking in food particles. The opposing theory disavows the likeness, maintaining that the egg grows by the dissolution of the cell walls between the egg and the cells contiguous to it, and that this process with its resulting conditions has no likeness to true amœboid activities.

The former view was first suggested by Balfour ('80) and upheld by Weismann ('83), Metchnikoff ('86), Brauer ('91) and conditions apparently supporting it have been described by many more recent writers. Doflein ('97) was the first to publish ob-

servations seriously opposing it. He described the growth of a coelenterate egg as taking place by the fusion or blending of the egg with its neighboring cells, and pointed out further, that vacuoles do not form about the engulfed cells in the case of those eggs described by the supporters of the theory, but only about their persistent nuclei. He concluded, therefore, that the amœba likeness was a misconception. Ciamician ('79) had previously described the enlargement of an egg by the coalescence of the egg and the cells surrounding it.

The present conception may perhaps best be indicated by quoting from Wilson ('00)¹ who states that the egg cell in coelenterates may move actively about in the neighboring cells like an amœba and "in such cases (hydroids) the egg may actively feed upon the surrounding cells, taking them bodily into its substance, or fusing with them and assimilating their substance with its own. In such cases (*Tubularia*, *Hydra*) the nuclei of the food cells long persist in the egg cytoplasm forming the so-called 'pseudo-cells' but finally degenerate and are absorbed by the egg."

It does not seem necessary to assume any active amœboid propensities on the part of the egg of *Hydra* to account for the processes concerned in its growth. That the egg in its early history is entirely devoid of any similarities to an amœba is evident from the account given above of its growth at that time. The coalescence of the primitive ova is surely not comparable to any amœbic activity. Nor do the ova migrate from one part of the body to another as in the case of certain hydroids. The growing egg does not, at first, at any rate, feed upon the surrounding cells after the manner of an amœba engulfing food particles. That the surrounding cells become a part of the egg is true, but the process by which the union is accomplished is simply a coalescence of a group of cells to form a larger mass over which a single nucleus comes in time to hold sovereignty. Possibly but one nucleus is functional from the beginning of the process. Possibly one cell is at all times the controlling factor in all of the processes, but this is difficult to prove.

Nor does it seem necessary to assume any active amœboid properties on the part of the egg in order to account for the fact

¹ "The Cell in Development and Inheritance," p. 150.

that cells are taken bodily into it. Indeed, the facts already set forth are not at all comparable to amoeboid activities. Some cases were found in which it would appear that the nourishing cells over a limited area had been transformed into pseudo-cells even before entering the egg. Occasionally, isolated cases of such changes were found at a considerable distance from the egg. Again, the protoplasmic mass resulting from the processes above described is under a certain pressure due to the tension of the ectodermal layer by which it is covered, and would therefore tend to flow into intercellular spaces and often simulate active amoebic engulfments by partially surrounding a cell. It seems more probable that the egg by some chemic influence causes a profound change to take place in the cells which surround it. Possibly the regressive changes, including the formation of the pseudo-cells, is due to this cause. The amoeboid form of the egg does exist but perhaps due in part, at least, to the causes suggested above. Just before breaking through the ectoderm, in all cases examined, the egg had appropriated all of the nourishing cells between it and the ectoderm. Rarely, there are groups of cells on either side of the egg which have not been appropriated. The rounding off process described for the eggs of cœlenterates is not due entirely to any activity on the part of the egg of *Hydra* to effect such a form, but is the result, in large part, of the consumption of all of the available nourishing cells within the germinal area so that a more or less regular outline results. That undoubted similarities between the growing egg and an amoeba do exist cannot be denied, but I am persuaded that these likenesses are frequently too widely applied and lead to misconceptions.

SUMMARY.

1. The egg begins its growth by the coalescence of a group of the primitive ova; this process is frequently attended by a peculiar nuclear degeneration. One of the nuclei from the group becomes the functioning nucleus; the others either disappear in the cytoplasm or are transformed into pseudo-cells.
2. The egg thus beginning its growth continues to increase in size by two processes: (a) By the disappearance of the cell wall between it and neighboring cells attended by a regressive

change in the nucleus which later becomes scattered and absorbed in the cytoplasm. (δ) By a union with neighboring cells which later, either in whole or in part, become transformed into the so-called pseudo-cells. This process may consist in the engulfment of whole cells.

3. The pseudo-cells are metamorphosed whole cells, nuclei or nucleoli, in which certain characteristic changes have been brought about. These changes are doubtless produced by a chemic influence from the egg.

This paper was undertaken at the suggestion of Dr. C. W. Hargitt. I desire to express my sense of obligation to him for his valuable criticisms and helpful suggestions.

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EXPLANATION OF PLATE I.

FIG. 1. Longitudinal section through a portion of the germinal area. The character of the cells is well shown, as also the metaplastic bodies frequently occurring in these cells. The double character of the nucleoli is noticeable. That they are composed of different material is evident from the difference in staining capacity. A nematocyst (*nem.*) is shown imbedded in the mass of cells. $\times 975$.

FIG. 2. A group of the primitive ova in which the regressive changes are just started. In the cell marked (*a*) the chromatin is retreating toward the periphery of the nucleus; in that marked (*b*) the spireme is formed. The cells are a little larger than the other primitive ova which are shown in the upper right hand corner. $\times 850$.

FIG. 3. The distal portion of a growing egg in which the outline of an engulfed cell is clearly shown. The cell-wall appears as a well-defined line. $\times 850$.

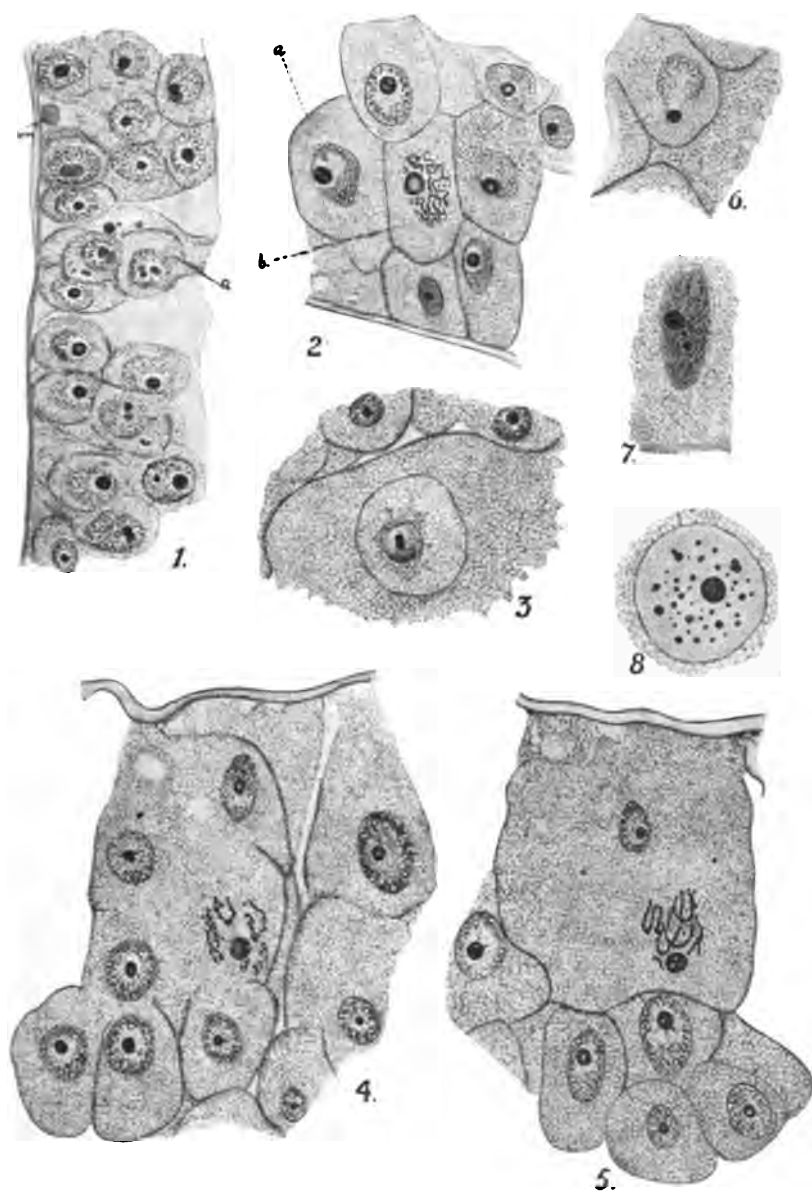
FIG. 4. The fragmentation of the nucleus of one of the cells contributing to the mass of the egg. The cell-wall has in part disappeared, while the coalescence with a neighbor cell has begun. The nuclear membrane has dissolved and the chromatin filaments are being scattered. Vacuoles are conspicuous in the nucleolus. $\times 850$.

FIG. 5. A condition not quite so far advanced as that in Fig. 4. Several metaplastic bodies are apparent. In the cells bordering on the egg, the regressive nuclear changes have begun. $\times 850$.

FIG. 6. A nourishing cell bordering on the egg. The nucleolus has broken through the nuclear membrane. Within, the broken chromatin filaments are plainly visible. $\times 650$.

FIG. 7. A young nucleus showing the single large nucleolus and several small nucleoli. Chromatin filaments are evident. $\times 650$.

FIG. 8. An older nucleus in which the nucleoli are very numerous. There are no traces of chromatin filaments whatsoever. $\times 650$.



EXPLANATION OF PLATE II.

FIG. 9. A drawing of a part of a growing egg showing the nucleus in the process of shortening from the elongated form. The character of the cells outside of the egg itself is typical. In one of them the regressive changes are well under way, the nuclear membrane having disappeared entirely, and the chromatin filaments set free in the cytoplasm. One of the very large cells, probably resulting from the coalescence of two cells, is likewise shown. Darkly staining lines in chromatin material indicate the probable fate of the nuclear stuff.

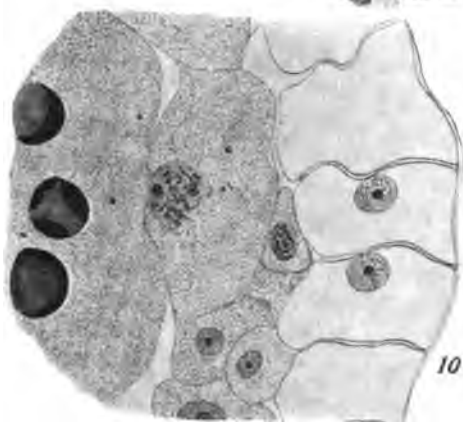
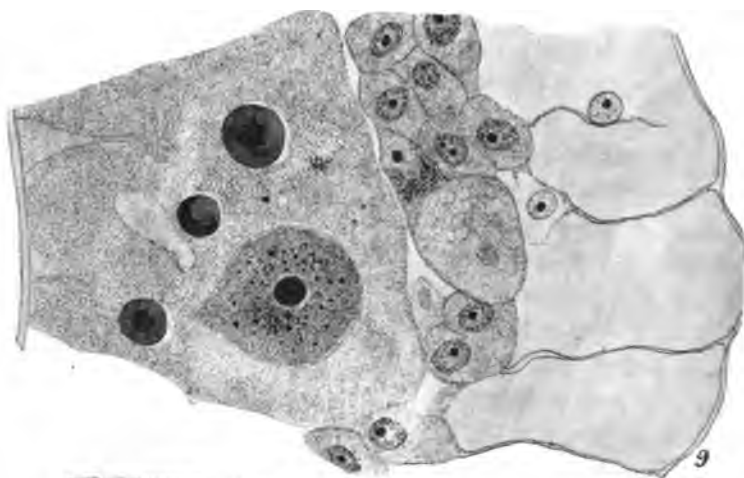
Within the nucleus of the egg are a large number of nucleoli and a single large nucleolus. In the cytoplasm is a tangle of filaments evidently the remnants of a cell whose mass has been added to that of the egg. Several metaplastic bodies are also visible. $\times 650$.

FIG. 10. A portion of a growing egg and a group of cells upon its periphery. The very large cell, probably derived from the fusion of two or more, is about to unite with the egg mass. In its nucleus the regressive changes are well advanced. In the small cell outside of this large one, the chromatin has assembled in the spireme, preparatory to union with the large cell. $\times 650$.

FIG. 11. A part of an egg in which occurred one of the very large pseudo-cells. Three thickened and darkly staining portions of its rim are noticeable. Along its edges, in two places at least, one can see the nucleoli from the cells contributing to the mass. $\times 650$.

FIG. 12. A portion of the ectodermal layer of a fully segmented egg showing the manner of outgrowth of the cells previous to the formation of the chitinous shell. $\times 650$.

FIG. 13. The beginning of the shell formation by the development of the chitinous layer outside of the ectodermal processes. $\times 650$.



EXPLANATION OF PLATE III.

FIG. 14. Distal portion of an egg at about the time of its breaking through the ectoderm, and previous to the process of maturation. The nucleus at this time becomes less darkly staining, and irregular in outline. The pseudo-cells are typical in that there are shown the variety of forms and sizes. $\times 650$.

FIG. 15. A nest of nucleolar bodies which have evidently been derived from a single nucleus by the dissolution of the nuclear membrane. These stain with different degrees of intensity, and in double staining, in different colors, indicating their difference in composition. $\times 850$.

FIG. 16. *a*, section through a pseudo-cell showing the thin rim of darkly staining material and the nucleoli arranged within it. *b*, another section in which the peripheral disposition of the nucleolar masses is conspicuous. *c*, a pseudo cell in which the nucleoli are shown in the pole opposite the darkly staining one. In this case they take a distinct plasma stain when double stained. $\times 850$.

FIG. 17. Cross-section of a pseudo-cell probably derived from a nucleus. The peripheral disposition of the chromatin is apparent in this as in the other cases. $\times 850$.

FIG. 18. A pseudo cell which shows its triple origin. Three nuclear masses are apparent as also three separate areas which stain darkly. $\times 850$.

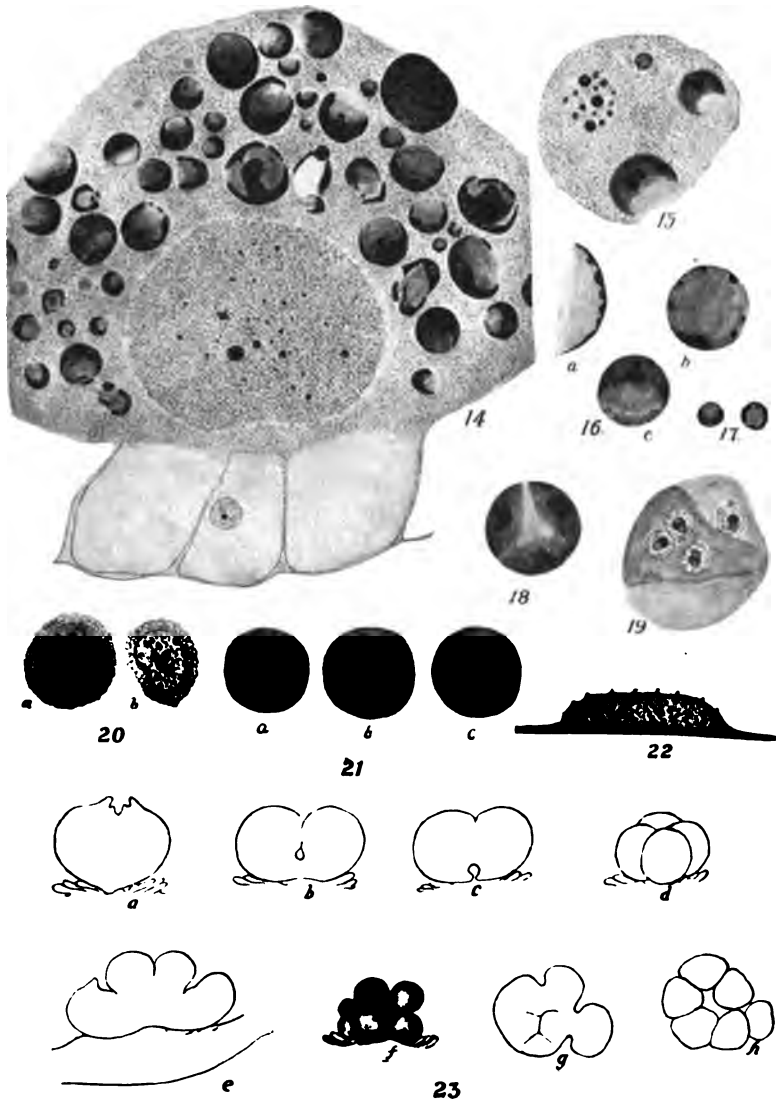
FIG. 19. A very large body found just outside of a growing egg, and in which were shown four well defined nuclei and traces of a fifth. Such conditions were found but once and may have been an artifact. However, the very large size of some of the pseudo-cells indicates that such large masses of coalesced cells frequently occur. $\times 850$.

FIG. 20. A number of nuclei as found in some of the cells outside of the egg. There were in some a number of nucleolar bodies which were not of true chromatic origin but apparently food masses within the nucleus. They were of a yellowish color. These were not found in all cases, but those sections on which they were found were apparently well fixed and stained. Possibly their presence depended upon certain metabolic processes. $\times 850$.

FIG. 21. Three stages in the metamorphosis of a whole cell into a pseudo-cell. In *a*, the outline of the nucleus is still evident and traces of the chromatin filaments are also visible. The nucleoli are peripherally arranged within the nucleus. In *b*, the nucleolar bodies have become massed together and the hemisphere of the cell in which they lie is slightly more darkly staining than in the previous figure. In *c*, the transformation is complete. The nucleolar mass is visible, but its containing hemisphere is now very darkly staining, the boundary of the stained being sometimes sharply defined from the unstained portion. $\times 650$.

FIG. 22. One of the flattened short spiculated eggs; drawn from a section.

FIG. 23. A series of drawings of a segmenting egg. *a*, *b* and *c*, the beginning, middle and ending, of the first cleavage. *d*, the four-celled stage. Up to this time cleavage is regular, total and equal. *e*, drawing showing the irregularity of the later cleavages. In this case the cleavage lines were not entirely visible. *f*, *g* and *h*, later stages, showing more pronounced irregularities.



EXPLANATION OF PLATE IV.

FIG. 24. Equatorial section through the egg segmented to the eight-celled stage. The cleavage cavity and protoplasmic bridges are well shown.

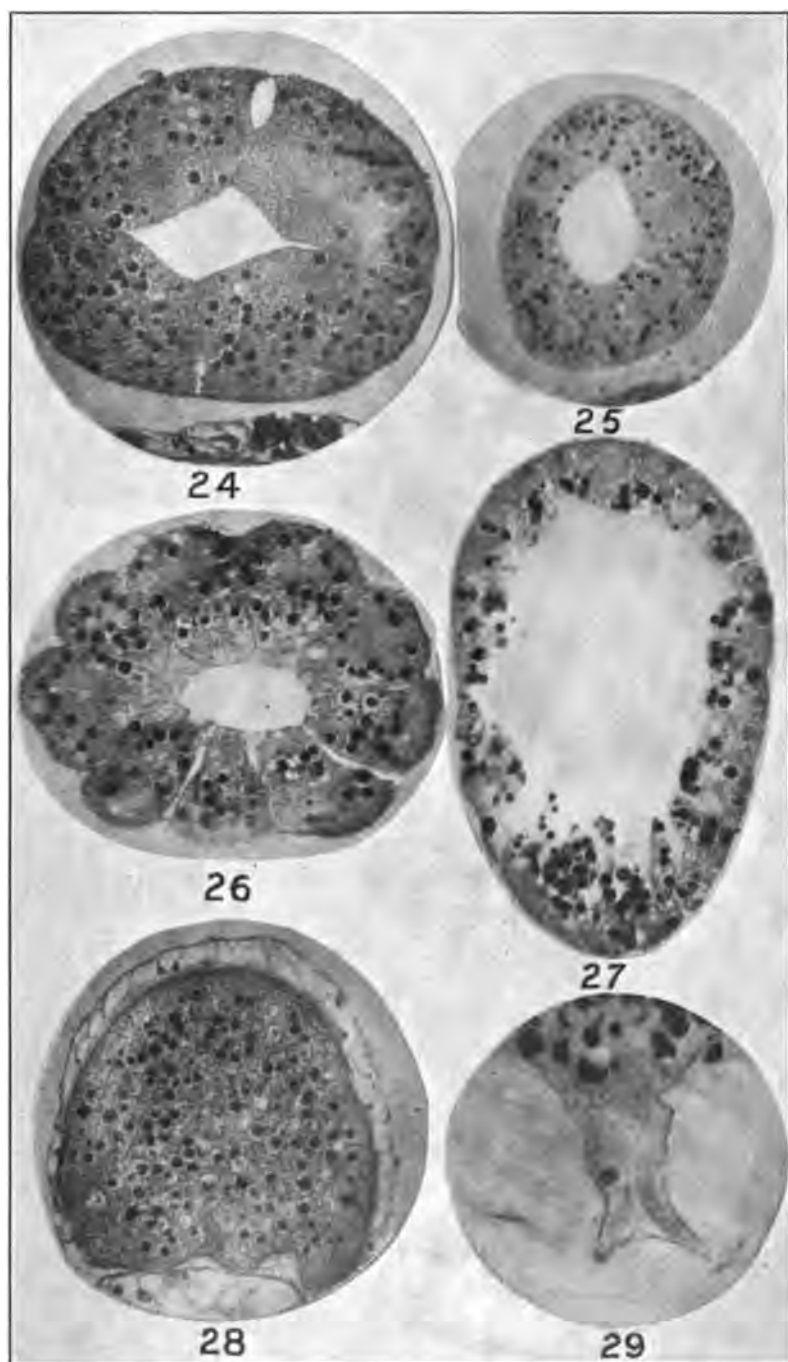
FIG. 25. Similar section through the egg at a little later stage. The cleavage cavity is more definite and a variation in the size of the blastomeres is noticeable.

FIG. 26. Section through the segmented egg at a more advanced stage. In this case the process seems to have been unusually regular. The blastomeres are apparently of nearly uniform size, and regularly arranged.

FIG. 27. Segmentation carried to the completion of the hollow mass of cells just before the beginning of the formation of the primitive entoderm cells. Some of the cells are noticeably larger and longer than others.

FIG. 28. The growth of the egg completed. The first polar body is to be seen just beneath the ectodermal covering. The egg membrane is also visible as a thin slightly shaded area beneath the ectoderm.

FIG. 29. The protoplasmic process from the ectodermal cells about which the spiculate structures are formed. The chitinous envelope is already forming. The dark spot in one of the processes is not a pseudo-cell, but a piece of foreign matter in the preparation.



SOME NOTES ON THE ANATOMY OF THE THYROID GLAND IN SELACHII.

A PRELIMINARY COMMUNICATION.

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During some investigations into the relation of the blood supply to the glandular masses of the thyroid, pursued at the Marine Biological Laboratory at Woods Hole during the summer of 1905, I found it necessary to first inquire into the anatomy and anatomical relations of the thyroid gland in the Selachii. The anatomical literature yielded such scant results, both text and illustrative references being only causal, that I deemed it of some possible service to future investigators to present a short account of the results obtained.

The thyroid gland of the dogfish (*Mustelus Canis*) was exposed by the following process: A median longitudinal incision was made through the integument from the inferior mandible to the anus, care being taken not to injure the underlying structures immediately beneath, and closely adherent to the integument, we come, in the inframandibular and hyoid region, to the transverse fibers of the constrictor pharyngii muscle. An incision through this comparatively thin sheet of muscular tissue exposed the coraco-mandibular muscle, whose fibers, lying parallel to the body axis, were then severed by a transverse incision 2.5 to 4.5 cm. from its insertion; the anterior end was then reflected to its mandibular attachment. This exposed the thyroid gland, lying just behind the posterior border of the mandible, and resting upon the coraco-hyoid muscle whose fibers parallel those of the coraco-mandibular muscle which is the more conspicuous because of its size and compactness.

The thyroid gland is easily recognized by its shape and color, it being typically shield-shaped, the convex border forward, and having cells which carry a pronounced amount of yellowish pig-

ment of an almost orange hue. The organ is almost sheet-like in its thinness. A dogfish of 115 cm. had a thyroid gland measuring antero-posteriorly 19 mm., from side to side 42 mm., and in thickness 1 mm.; it weighed 502.5 milligrams.

The thyroid gland is approximately unilobular, yet shows some evidence of beginning lobulization. In addition to an almost separate posterior portion it often has in its main body certain indications of a tendency to further lobular subdivision formed by interruptions of the glandular tissue which are filled in by septa or trabeculae of loose connective tissue. There may be three incisures of this sort, all beginning at the posterior border of the main portion of the gland and extending, almost at right angles to the border, into the glandular substance. The most



FIG. 1. Outline of the thyroid gland of a dogfish as viewed from the ventral surface, showing the distribution of its arterial supply. The dotted lines indicate an area over which the thyroid substance was deficient, *a*, right (dorsal) thyroid artery; *b*, left (ventral) thyroid artery.

constant of these fissures, and the largest, is situated in the median line; a smaller indentation is often observed on either side of this primary one, almost parallel to it, and so placed as to be approximately equidistant between the primary fissure and the lateral border of the gland.

The smaller fissures may vary in extent, but the one on the right of the median line is nearly always shorter than that on the left. This difference is readily understood by a reference to the anatomical relation of the right thyroid artery to this portion of

the gland. The indentations and consequent traces of lobulization conform closely to the areas of distribution of the main vessels. The lower, nearly isolated portion of glandular tissue is supplied by a branch from the right mandibular artery which enters the gland on its ventral aspect. The partially detached portion of tissue is placed close to the point of entrance of this vessel and is therefore supplied by the right thyroid artery and not by the left, the vessel entering at the free end of the partially detached lobule; on the left this lobule is continuous with the body of the gland but does not, as one might expect, derive its blood supply therefrom. The right thyroid artery also sends an anterior branch to supply the right posterior third of the main portion of the organ. The remainder of the gland is supplied by a corresponding vessel, a branch of the left mandibular artery, whose area of distribution is much more extensive than that of the right thyroid artery; it enters the dorsal surface of the thyroid gland.

The arterial connections were traced with the aid of injections of India ink into the conus arteriosus or into the cardiac ventricle of a freshly opened fish by means of a hypodermic syringe having a needle of very fine caliber. The continued contractions of the ventricle were sufficient to auto-inject the vessels under consideration.

AUTOTOMY IN HOLOTHURIANS.¹

A. S. PEARSE.

I. INTRODUCTION.

It seems remarkable that any animal should make a regular practice of casting off parts of its body, and it is not strange that autotomy has long been a subject of more than passing interest to the scientific world. Although this phenomenon is present in a variety of animals it has been most fully studied in the arthropods and echinoderms. In the first of these groups the nervous control of the reflexes concerned has been investigated (Drzewina, :07; Morgan, :02), and there seems to be no doubt that autotomy may occur as a result of stimulation which exerts no direct mechanical strain on the parts thrown off (Torre Bueno, :08). It is even maintained to be a psychic phenomenon in some cases (Pieron, :07) which is due to conditions within the animal itself. Many echinoderms are known to break off portions of the body as a result of external stimulation (Lang, '96), but, to my knowledge, no studies directly concerned with the nervous control of such reactions have been made.

The experiments described in this paper were performed upon the two common holothurians which are found at Woods Hole, *Leptosynapta inhaerens* (O. F. Müller) and *Thyone briareus* (Leseur). The object of the work was to discover the relationship of the central nervous system to the reflexes involved in autotomy. Evidence was obtained in two different ways. In one method of experiment various chemicals were injected into the body cavity, and their effect upon the general behavior of the animal was observed in connection with the phenomenon of self-mutilation. In another series of experiments animals were cut in two and the subsequent reactions of the two ends were compared. In this way evidence as to the importance of the nerve ring was obtained.

¹ Contributions from the Zoological Laboratory of the University of Michigan, No. 127.

II. MECHANISM OF AUTOTOMY.

As Lukas (:05) has pointed out the place where self-mutilation takes place is usually determined definitely by the structure of an animal, and in the two genera under consideration the process is quite different. *Leptosynapta* constricts off pieces at the posterior end of the body (Fig. 1) until there is often only a small anterior fragment remaining. Such fragmentation is brought about by the strong local contraction of the circular muscles which pinch the body in two.

Thyone never constricts off pieces at the posterior end. In autotomy the body-wall breaks open just behind the calcareous

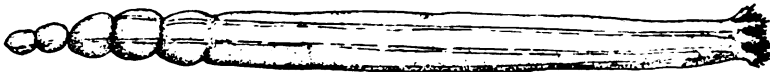


FIG. 1. *Leptosynapta inharens*, showing method of autotomy.

ring, at the point indicated by *b* in Fig. 2, and the visceral organs are thrown out. If the body is in the position shown in the figure, however, there will be no autotomy for the contraction of the circular muscles at *c* forces the calcareous ring (*c.os.*) back into the body. But if the tentacles are extended and the calcareous ring is pushed forward a break may occur at *b*, as a result of the strong contraction of the circular muscles at that point, and visceral organs are forced out. The calcareous, water-vascular and nerve rings are thus ejected from the body, together with the tentacles (*t*) and more or less of the alimentary canal (*s, i*). Whether this autotomy takes place or not depends upon the breaking of the inner branch (*l.m.2*) of the longitudinal muscle bands (*l.m.*), whose normal function is to retract the calcareous ring (*c.os.*). When the strain brought about by the contraction of the circular muscles (*c.m.*) becomes too great these inner bands are torn asunder, usually at the point *x*.

III. DEPENDENCE OF AUTOTOMY ON THE PRESENCE OF THE ANTERIOR PORTION OF THE BODY.

In *Leptosynapta* (Fig. 1) the manner of the fragmentation of the body points to an influence which perhaps comes from the circumoral nerve ring. Pieces are always constricted off from the body at the posterior end and fragments thus separated do

not again break up, as is the case in the arms of some ophurids. Furthermore, if an individual is cut in two in the middle of the body the posterior half does not constrict off pieces, though it undergoes irregular contractions and constricted rings are often formed. The anterior half however will continue to fragment at its posterior end. The results are the same if an animal is cut in two in such a way that the posterior piece contains two thirds or three fourths of the body.

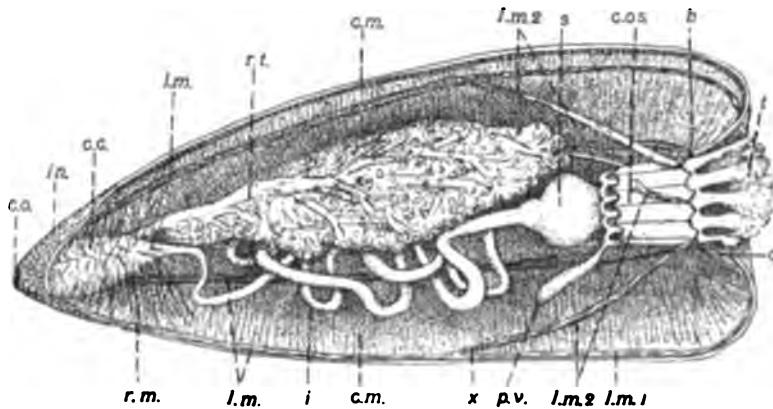


FIG. 2. *Thyone briareus*. The right side of the body-wall as well as some of the dorsal portion have been removed. The ampullae and mesenteries are not shown. *b*, point where body wall is ruptured in autotomy; *c*, region where circular muscles are effective in preventing autotomy; *c.c.*, cloacal chamber; *c.m.*, circular muscles; *c.o.*, cloacal opening; *c.o.s.*, calcareous ring; *i*, intestine; *i.n.*, integument; *l.m.*, longitudinal muscle bands; *l.m.1*, outer branch of longitudinal muscle bands; *l.m.2*, inner branch of longitudinal muscle bands; *r.m.*, radial muscles which dilate the cloacal chamber; *r.t.*, respiratory tree; *s*, stomach; *t*, tentacles; *x*, weak point in inner branch of longitudinal muscle band.

Thyone's mutilating reflexes always involve the loss of the nerve ring and the remaining portions of the body do not undergo further fragmentation but ultimately regenerate the lost parts. As in *Leptosynapta* there is no autotomy when the portion of the body which contains the nerve ring is absent.

IV. EFFECT OF CHEMICAL SUBSTANCES ON AUTOTOMY.

In order to ascertain whether substances which increased the general excitability would induce a corresponding increase in the number of individuals manifesting autotomy, certain drugs and

chemicals were injected into the body cavities of two hundred and forty-six holothurians. *Thyone briareus* was the only species used for these experiments, and the animals selected were of medium size, measuring from five to eight centimeters in length. As the normal reactions of this species have been described in a former paper (Pearse, : 08), they will not be considered in detail here. Twelve individuals were experimented upon at one time, and they were placed in pairs in six finger bowls which contained sea water. The substance to be tested was injected into the body cavities of ten individuals by means of a hypodermic syringe. The two remaining animals were pricked with a needle without having anything injected into them and served as a control. Observations extended over a period of twenty-four hours in each case. Experiments were started in the morning and observations were made at intervals during the same day. The condition of the animals on the following morning was also recorded.

The results of the experiments are set forth in Table I. A volume of distilled water which exceeded that of any of the other substances used was injected into ten individuals and they were apparently unaffected by it. It was therefore assumed that the effects obtained in the other experiments were due to the specific substances which were injected.

Substances like acetic acid and clove oil, which were apparently highly irritating and caused the most intense contractions of the muscles of the body-wall, did not bring about the ejection of the visceral organs. Nor were drugs like codene and atropine, which caused violent peristaltic waves of contraction to pass over the body, any more potent in inducing autotomy. The same may be said of sodium chloride, atropine and clove oil, although the injection of any of these substances was often followed by a waving of the oral tentacles to perform "feeding" movements, thus bringing about favorable anatomical relations for autotomy. All the reactions induced by the substances mentioned indicated violent stimulation or great bodily activity, but none of them produced any increased manifestation of self-mutilation.

The injection of strychnine was followed by the largest percentage (35) of cases of autotomy and methylene blue came next (22 per cent.). Strychnine apparently caused a great increase in

TABLE I.

SHOWING THE EFFECTS OF INJECTING CERTAIN SUBSTANCES INTO THE BODY CAVITY OF *Thyone briareus* (Leseur).

	Strength of Solution.	Amount Injected in c.c.	Number of Individuals Used.	Ejected Viscera.	Violent Peristaltic Contraction Waves.	No Peristaltic Waves.	Tentacles Extended.	Body Wall Puckered from Contractions.	Very Active.	Stopped Respiratory Movements.	Normal after 24 Hours.	No Response to Pricking with Pin.	Died.
Acetic acid.....	10 %	.1-25	20			20	20		20				20
Alcohol, ethyl.....	95 %	.77-3.85	10								2		6
Atropine.....	?	?	10		10		5 ¹				10		
Chloretone.....	Sat. sol.	.77-3.85	10	2			2 ¹				8	4	2
Chloroform.....	Conc.	.77-3.85	10					10	10			10	10
Codene sulphate.....	1 %	1-2	10	1	10		5 ¹	10	10		9	4	
Clove oil.....	Conc.	.1-2	20			10	11 ¹	6				20	20
Curare.....	Powder	?	20								20		
Ether.....	Conc.	.77-3.85	10	1		10				5		5	10
Magnesium chloride..	20 %	.77-3.85	10								6	4	
Magnesium sulphate..	20 %	.77-3.85	10			6					4	6	
Methylene blue.....	2 %	.77-1.54	18	4		18	1 ¹			18			18
Morphine sulphate...	1 %	.5	10								10		
Nicotine.....	.05 %	.15-3.85	20	2		20				8	9		11
Sodium chloride.....	10 %	1.55	10				5 ¹				10		
Strychnine.....	Powder	?	20	7			1 ¹		20				
Sugar.....	50 %	.77-3.85	9				1 ¹				7	2	
Turpentine.....	Conc.	.77	10	2			2 ¹				10		
Water, distilled.....	Conc.	5	9								9		
Total.....			246	19	20	84	33	46	30	61	114	55	97

the general activity without causing the reactions to become abnormal. The tube-feet over the whole body were waved actively back and forth, and both the peristaltic contractions of the body-wall and the respiratory movements were strong and frequent. Methylene blue, on the other hand, caused a complete cessation of such normal activities as respiratory movements and peristaltic waves; the body-wall and tube-feet were strongly contracted. This substance caused death in every instance.

Autotomy took place under quite diverse conditions in the two cases and cannot, in the light of these experiments, be affirmed to result from either "over" stimulation or extraordinary activity alone. In my opinion it may better be ascribed to what we may call a "structural accident," and it may occur when any combi-

¹ From June 22 to July 5, 1909, many individuals were ejecting eggs or sperms from the genital papilla. During this process the tentacles were extended and these reactions in the table may have been due to the physiological condition of the individuals rather than the effects of the substances injected.

nation of conditions causes the inner branches of the longitudinal muscles to be broken.

V. GENERAL CONSIDERATIONS.

It is apparent from the experiments which have been described that autotomy is not the result of any single factor which can be easily controlled, at least in the two species of holothurians studied. It is of more uniform occurrence in *Leptosynapta* than in *Thyone*, a fact doubtless due largely to the structural differences between them. Clark (:01, p. 25) in speaking of *Leptosynapta* says, "Autotomy is not normal or defensive, but is due entirely to pathological conditions." Some holothurians (*e. g.*, *Cucumaria*), however, are said to multiply regularly by constricting the body in two in the middle (Lang, '96). Self-mutilation probably occurs in nature as a result of such conditions as foul water or too high temperature. The process might be beneficial to either of the two species under consideration when the environmental conditions were unfavorable for existence. By throwing off a portion of the body the total amount of metabolism necessary would be decreased. An individual might thus survive until conditions were again favorable and the lost parts could then be regenerated.

It can be affirmed that autotomy is apt to occur as a result of unfavorable stimulation in both *Leptosynapta* and *Thyone*. In the former genus it is induced uniformly and regularly when the proper conditions arise, *e. g.*, lack of sand for burrowing, foul water. It might in this case be considered to be a definite reflex and evidence has been given (II., p. 43) which lends some support to such a view. The fact that the constrictions which pinch the body in two are formed only when a region is connected with the anterior portion of an animal shows that some influence which comes from the anterior end is essential. It seems reasonable to suppose that such an influence comes from the only central nervous system which holothurians possess—the nerve ring. Autotomy apparently involves a reflex in this case which is similar to those found in arthropods (Drzewina, :07; Morgan, :02; Torre Bueno, :08).

When we turn to *Thyone*, however, autotomy is by no means

so uniform and regular in its occurrence. Under the best of conditions it appears in only 35 per cent. of the possible cases. As has been stated, the phenomenon depends in this genus upon the breaking of certain muscles and the rupture of the body-wall. In some individuals there seems to be an actual struggle between the different parts of the body, and the observer is often in doubt for a time whether the longitudinal muscles will pull the visceral organs back within the body or whether the intense contraction of the general body muscles will eject them. One is easily led to believe that the activities of different parts are not well correlated, and that the part which gets the initial advantage gains control.

As the mechanism for ridding the body of certain parts is quite different in the two genera under consideration, it is not possible to compare them directly. The process is more stereotyped in *Leptosynapta*. In the opinion of the writer, the words which were just quoted (p. 47) from Clark (:01) in regard to that genus could better be applied to *Thyone* and it seems improbable that autotomy is an important factor in the daily life of the members of either genus. Nevertheless, it is easy to conceive that a process of this kind, which first arose as a result of certain structural and physiological peculiarities, might in time give rise to a process which would become advantageous to the species. In fact, this condition of affairs seems to have come about in some holothurians (e. g., *Cucumaria*) which multiply by constricting the body in two in the middle.

The tendency toward autotomy is apparently marked in the holothurian line. It is a phenomenon which is accompanied by remarkable powers of regeneration and in some species it has become a beneficial process, but in others it is comparatively unimportant, if not actually harmful.

VI. SUMMARY.

1. In *Leptosynapta inherens* autotomy occurs regularly when unfavorable conditions of environment arise. Experiments with *Thyone briareus* did not show more than 35 per cent. of cases of autotomy, even under the most favorable circumstances for its occurrence.

2. The mechanism involved in autotomy is quite different in the two genera. *Thyone* breaks the longitudinal muscles and throws out the visceral organs at the anterior end. *Leptosynapta* constricts off pieces at the posterior end of the body.

3. Portions of the body which have been separated from the anterior region, which contains the nerve ring, do not show autotomy.

4. Strong stimuli which bring about a very active or a strongly contracted condition do not always induce autotomy.

UNIVERSITY OF MICHIGAN,
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THE HEREDITY OF EYE COLOR AND HAIR COLOR IN MAN.

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Since the present study was begun several papers have appeared dealing with the subject matter of our investigations. Much of our data corroborates the conclusions expressed in these papers. In some cases the facts seemed to warrant some differences of opinion in regard to certain theoretical matters, and it was therefore deemed advisable to present the data obtained and the conclusions that appeared to be deducible therefrom.

The data were collected by sending out printed forms with headings under which to designate the eye color and hair color of each parent, grandparent and child and the age and sex of each of the children of the family. If the children came from parents one or both of whom were married more than once the exact relation of the various individuals was stated. Eye color was classed as blue, green, gray, hazel, brown and black, and hair color as yellow, light brown, dark brown, black, red and auburn. The printed forms were distributed mainly to the students of the University of Wisconsin with the request that so far as possible they fill them out in consultation with their parents during the holidays. Fairly complete data were received from 71 families including 406 individuals. While the data are not extensive nor free from sources of error they are, I think, sufficiently complete and reliable to enable one to draw several conclusions which have a high degree of probability.

EYE COLOR.

The color of the eyes is due to the factors causing blue, a dark brown granular melanin pigment, and in such cases a yellow pigment which is possibly a lipochrome. Blue is often darker in the young. "The darker shades of blue," according to Hurst, "are apparently due to the greater delicacy and transparency of the fibrous tissues of the iris through which the posterior pigment

is seen while the lighter shades of blue and the coarser grays seem to be due to the greater coarseness and opacity of the same tissues." These tissues "become coarser with age and young children with dark blue eyes may mature into adults with light blue, blue or gray eyes."

The various shades from light brown to black are due to different amounts of dark pigment deposited in the iris, blue eyes being those in which dark pigment is absent. Green is produced by a small amount of yellow pigment with blue or black. Yellow pigment, however, is of minor importance compared with the other color factors. Eyes commonly classed as gray may contain a small amount of dark pigment with the blue or blue and yellow or they may be due to a fine mottling of green and blue. Eyes are very commonly mottled in various ways, and they are frequently "ringed," the darker color being more dense around the margins of the pupil.

It is evident that eye colors cannot be divided into sharply defined classes. All sorts of intermediate shades occur as well as irregularities in the distribution of pigment over the surface of the iris. Color classes, therefore, are largely arbitrary categories. Perhaps, as the Davenports suggest, the most natural grouping is the one based on the presence or absence of melanin pigment. Black to light brown would fall into one class. Blue and green would fall into another while the grays would be divided between the two. There are, however, all grades in the amount of melanin pigment present and whether there are cases in which it does not exist in minute traces may reasonably be questioned. Hurst has divided eyes into the simplex and duplex types; in the former dark pigment occurs only in the posterior coat of the iris; in the latter in both coats. As a rule the darker colors belong to the duplex type, the lighter colors to the simplex type. This relation suggests that the difference may be due mainly to the amount of general pigmentation, the amount of pigment when small being deposited in the posterior coat and extending also into the anterior coat only when present in increased quantity. There is nothing which shows that these classes are not the result of a purely continuous series of variations.

Our results on the inheritance of blue eyes bear out in general

the conclusions of Hurst and the Davenports that blue behaves as a recessive to the pigmented condition. Blue $\times$ brown or black gives either brown or black, or brown or black with hazel, gray or blue which is the expected result if brown or black are heterozygotes. Blue $\times$ blue as a rule gives only blue. However the rule is not without exceptions. In one case a man with brown eyes was born of blue-eyed parents. Another instance was communicated by a gentleman who stated that both his parents had light blue eyes. Of their seven children all had blue eyes except one sister whose eyes were described as brown or dark hazel. Pearson gives one instance in which one member of a family of six children born of blue-eyed parents had dark brown eyes like those of the father's maternal grandfather.

In gray $\times$ blue matings, gray and blue were the usual result but brown eyes appeared in seven out of 72 offspring. In gray $\times$ gray matings brown eyes appeared once out of 17 offspring. Black eyes resulted in a few instances from brown $\times$ gray and brown $\times$ blue matings, but these cases may have been due to errors of classification, as brown eyes, especially if dark, are frequently described as black. Matings in which both parents have black or brown eyes may give either dark-eyed or light-eyed children. Thus black $\times$ black gave offspring with black, brown, gray and blue eyes. Matings in which one parent had dark eyes and the other light gave both dark-eyed and light-eyed offspring, but the proportion of the latter was considerably greater than in the previous case. In general we may say that the more darkly pigmented condition is dominant over the lighter, black over brown, brown over gray or blue, and gray over blue.

The inheritance of eye color in man, as is well known, is to a considerable degree alternative. How far it is Mendelian is a question rather difficult to answer on account of the intergradation of colors, limited knowledge of the ancestry of the families, and other causes. In man it is difficult to distinguish the pure dominants from the dominant-recessives; in fact it is impossible to do so with data covering only three generations. While it is probable that the blues are recessives there is no certainty in regard to the browns and blacks. Were blue-gray bearing

gametes present in equal numbers with the carriers of the darker colors we should expect to find light eyes in one fourth of the population. In a population in which over half the individuals fall into the light-eyed class it is evident that a much greater proportion than two thirds of the dark-eyed class are heterozygotes. In the 395 offspring tabulated the color of eyes was as follows: black 29, brown 110, hazel 3, gray 93, green 10, blue 150. It is safe to assume that much over three fourths of the dark-eyed individuals are heterozygotes but it is impossible to make more than a rough estimate of the ratio from the data at hand. In crosses of brown $\times$ blue we should expect half the offspring to be brown even if all the browns were heterozygous. If some of the browns were dominant the proportion would be greater. As a matter of fact we get 48 blacks or browns to 69 of the gray-blue class which is very different from the expected Mendelian ratio.

In crosses of brown $\times$ gray we get 30 of the dark class to 17 of the light which is more in accordance with the Mendelian expectation. In the black $\times$ blue crosses we get 30 of the dark color to 18 of the light which is very close to the preceding result. Black $\times$ gray gave 13 dark to 8 light.

Much of the data obtained may be interpreted as illustrating Mendelian inheritance but it is by no means certain that it should be so interpreted. There are, on the contrary, several cases which refuse to come under the Mendelian formula. There are indications that the inheritance is to a certain extent of the blended type. Crosses of brown or black with gray give a relatively greater number of browns than do crosses of brown or black with blue. Since gray frequently contains a certain amount of melanin pigment it is readily understood, if the inheritance is partially blended, why many more offspring should be raised into the brown category than in the crosses with blue. Again, compare the crosses of black $\times$ blue with those of brown $\times$ blue. In the former the proportion of blues is small, 4 out of 48, where the Mendelian expectation (if the blacks are predominantly heterozygous) is much greater. In the latter 46 out of 117 are blues. This can hardly be accounted for by any difference in the proportion of heterozygotes between the blacks and browns, which is

within the limits of probability. If the inheritance is partially blended we should expect that with increased pigmentation of either of the parents there would be an increased number of darkly pigmented children; and this we find. Inspection of Table I. will show many other cases which may be quite as readily interpreted as cases of blended inheritance as of mendelizing.

The principle of the non-transgressibility of the upper limit which is laid down by the Davenports represents only a very general tendency rather than a general law. Aside from the exceptions described Pearson records a family in which the two parents had light gray eyes, four children had eyes like their parents, while five others had black or brown eyes. De Candolle found that out of 257 individuals born of parents both of whom had gray or blue eyes 23 or 8.9 per cent. had brown eyes. I have met with one instance in which both eyes and hair were of a distinctly darker brown than they were in the darker parent. As we are not dealing with hard and fast unit characters but with different degrees of pigmentation it is not surprising that the eyes of children should occasionally be darker than those of the parents. This may account for some of the cases of apparent blends, but, in the light of our results on hair color, it is hardly probable that it can account for all.

HAIR COLOR.

Hair color, like eye color, is the result of more than one factor. There is a granular dark brown melanin pigment which causes variations in intensity from light brown or yellow to black according to quantity. There is also a diffuse reddish pigment which may cause variations from reddish yellow to dark golden. These two kinds of pigment are usually mixed in various proportions; auburn and chestnut brown for instance arise from a combination of the two. Both these kinds of pigmentation appear to vary continuously and independently. The reddish pigment is frequently obscured by the brown. It may appear in children of parents with dark brown or black hair, but does not occur in children of light-haired parents who have no red pigment. Our data on the inheritance of red are meager, but so far as they go they confirm the conclusions of the Davenports on the inheritance of this color.

The effect of age upon the color of hair is so great that any conclusions based on the hair color of children as compared with their parents is of comparatively little value. The color of hair changes most rapidly in early life, but there is a considerable change even after sixteen years of age. We have made a rough estimate of the change by compiling the relative frequencies of the three most common colors in children under sixteen, in the members of the third generation in our data over sixteen, in the parents and in the grandparents. In children under sixteen black forms 9.8 per cent. of the individuals. In the members of the third generation over sixteen it forms approximately 20 per cent. In the parents and grandparents it forms in each case 39.2 per cent. Brown is less frequent under sixteen. It is most common in the children over sixteen, but in the parents and grandparents it is less common on account of so many developing into black. The light browns steadily decrease in number

TABLE SHOWING THE RELATIVE FREQUENCIES OF THE THREE MOST COMMON VARIETIES OF HAIR COLOR AT DIFFERENT AGES :

	Children under 16.	Children over 16.	Parents.	Grandparents.
	Per Cent.	Per Cent.	Per Cent.	Per Cent.
Black.....	5 = 10	45 = 20	49 = 39.2	84 = 39.2
Brown.....	23 = 45.1	127 = 56.4	62 = 49.6	99 = 46.8
Light brown.....	23 = 45.1	53 = 23.5	14 = 11.2	31 = 14

SIMILAR TABLE COMPILED FROM THE DATA OF THE DAVENPORTS.

	Children.	Parents.	Grandparents.
	Per Cent.	Per Cent.	Per Cent.
Black.....	70 = 12.2	121 = 38.9	213 = 39.2
Brown.....	292 = 50	131 = 42.1	230 = 44.2
Light brown.....	212 = 36.9	59 = 18.9	99 = 18

with age. We have made a similar tabulation from the data given by the Davenports, although, since the ages of the children were not given, all of the third generation are treated together. In order to make the results more nearly comparable the browns and dark browns of the Davenports are classed as browns, and the light browns and yellowish browns as light browns. Their data show that black is over three times as prevalent in the parents as in the children while light brown is over twice as common in the children as in the parents or grandparents. The proportions of yellow, tow and flaxen are relatively greater in the

young as compared with the adults than light brown, tow occurring in twenty children and in none of the adults. The data agree with ours in showing little change between the parents and grandparents. It is evident that an investigation of the heredity of hair color under the assumption that the juvenile condition represents even an approximate record of heredity would be much like a study of the inheritance of stature from measurements of the height of school children.

Any conformability of the results thus obtained with Mendelian expectations, so far as ratios are concerned, means little. In fact if the ratios were approximately Mendelian before the effects of age were excluded they could not be Mendelian after the elimination of this factor.

TABLE I.

SHOWING THE NUMBER OF CASES OF THE VARIOUS KINDS OF EYE COLOR RESULTING FROM DIFFERENT MATINGS; BL, BLUE; BLK, BLACK; BR, BROWN; GN, GREEN; GV, GRAY; HZ, HAZEL.

Eye Color of Parents.	blk	br	gn	gy	bl	hz	Total.
blk × blk							
blk × br	4	8		1	4		17
blk × gn							
blk × gy	3	10		8			21
blk × bl	15	13		14	4	2	48
br × br		22			1		23
br × gn	1	3	1		1		6
br × gy	3	30	2	12	3	1	51
br × bl	3	45	6	17	46		117
gn × gn							
gn × gy							
gn × bl			1				1
gy × gy		1		11	5		17
gy × bl		7		28	37		72
bl × bl		1		2	49		52
Total	29	110	10	93	150	3	395

The data obtained (see Tables II. and III.) warrant us in concluding that in the inheritance of colors depending on the granular dark brown pigment the same general tendencies prevail as in the inheritance of eye color. Dark hair tends to be dominant over the lighter colors. If both parents have dark hair the children will be predominantly dark haired but a certain number of light-haired children may appear. If one parent is dark haired and the other light haired both dark-haired and light-haired chil-

dren may be produced, but the latter will be more abundant than in the previous case. Light-haired parents rarely produce dark-haired children.

In order to eliminate so far as possible the effects of age a separate tabulation (Table III.) was made of the relation of the hair color of parents and grandparents. As we have seen, barring the effects of turning gray, there is comparatively little average difference between these two classes. In order to secure as much data as possible we have combined the uncompiled data of the Davenports in relation to parents and grandparents with our own. As this material was classified somewhat differently from ours

TABLE II.

SHOWING THE NUMBER OF CASES OF THE VARIOUS KINDS OF HAIR COLOR RESULTING FROM DIFFERENT MATINGS; AU, AUBURN; BLK, BLACK; BR, BROWN; LT BR, LIGHT BROWN; RD, RED; YL, YELLOW.

Hair Color of Parents.	blk	br	lt br	yl	au	rd	Total.
blk × blk	36	12	5		1	1	55
blk × br	38	49	27	1	9	3	127
blk × lt br	6	21	10	1	3		41
blk × yl	1		1	1			3
blk × au	4	12	4	1	6		27
blk × rd	1	2			3		6
br × br	4	66	17		3		90
br × lt br		8	16	2	4	1	31
br × yl		1					1
br × au	1	5	6	1	1		14
br × rd		1					1
lt br × lt br		2	4				6
lt br × yl							
lt br × au		1	1		1		3
au × au					1		
Total.	91	180	91	7	32	5	406

we have attempted to bring it so far as possible within the same categories; the browns and dark browns of the Davenports we have called browns, the yellow and golden colors have been grouped as yellow, the yellowish browns and light browns as light browns, the dark reds and auburns as auburns, and the blond and flaxen types as flaxen. The number of cases which might be classified differently from the grouping employed is quite small and could not vitiate any conclusions we have attempted to draw from the data.

It may be seen that in crosses of black and black that many cases of lighter hair make their appearance, a result that might be explained on the assumption that a considerable proportion of the blacks are heterozygous. In the crosses of black and brown we should naturally expect to get a larger percentage of browns and lighter colors, and this we find. In the crosses of black with light brown the proportion of browns and lighter colors is very different from the Mendelian expectation in the light of the preceding results. If all the blacks were heterozygous, the blacks should constitute 50 per cent. of the product instead of much less than a third; while, since some of the blacks may be safely assumed to be homozygous, the black-haired offspring should

TABLE III.

SHOWING THE INHERITANCE OF HAIR COLOR OF PARENTS FROM GRANDPARENTS

Hair Color of Grandparents.	blk	br	lt br	yl	au	rd	flxn	Total.
blk × blk	49	18	5		1	2		75
blk × br	46	34	6		3			89
blk × lt br	10	17	7		1		1	36
blk × yl			1					1
blk × au	4	2		1	2			9
blk × rd	6	2			2	2		12
blk × flxn		2					2	4
br × br	10	55	14	2		3	1	85
br × lt br	8	18	21		2	2		51
br × yl	2		1					3
br × rd	3	5	1			1		10
br × flxn		2	2					4
lt br × lt br	1	3	11					15
au × au					1			1
flxn × flxn				1			6	7

exceed 50 per cent. Crosses of browns with browns give a larger number of browns than result from crosses of browns and light browns; but it is noteworthy that a considerable proportion of black-haired individuals result from both these unions.

While the data indicate that the inheritance of hair color in man is, to a certain extent, alternative, it certainly does not justify us in concluding that it is Mendelian. That it is to a certain extent of the blended type is indicated by the fact that crosses of black with light brown yield a much less proportion of blacks and a greater proportion of browns than do crosses of blacks with brown. If black were a typical Mendelian dominant it should occur in

equal proportions in both cases. In fact many of the results obtained by the Davenports and ourselves may be interpreted quite as readily as blended inheritance as anything else.

It is a rule laid down by the Davenports that "in the midst of varying degrees of melanic pigmentation the intensity of melanic pigmentation never exceeds that of the more intense parent." This is a general rule borne out also by our data; but it is by no means universal. While the data compiled by the Davenports show few or no cases in which this rule is plainly violated, I find upon examining their data on the relation of parents and grandparents, a considerable number of exceptions. Apparently no account of these relations was taken, the data concerning the grandparents being used only on account of the light it might throw on the probable constitution of the gametes. In one respect these data are more valuable than what was used, which

TABLE IV.
SHOWING INHERITANCE OF THE CORRELATIONS OF EYE COLOR AND HAIR COLOR.

Combination.		No. Families in which Occurs.	Inheritance as Correlated Variation.	Appeared in Generation I. or II. but not Transmitted as Correlated Variation.	Appeared in Generation III. only.
Hair.	Eye.				
blk	blk	23	13	12	
blk	br	28	37	10	
blk	bl	17	8	11	
blk	gy	14	5	9	
br	br	23	40	5	
br	bl	37	55	9	2
br	gy	24	22	12	
lt br	bl	27	18	7	14
rd	bl	4	3	2	1
au	bl	8	13	2	1
au	br	6	0	3	4
lt br	gy	19	6	5	15

was derived from the members of the third generation, as the effects of immaturity are mainly eliminated. In their table 7a, the rule is violated in 5 out of 13 families, black hair in the father of the Sim family coming from parents both of whom had light brown hair. In the other tables given, less frequent deviations are found but they are sufficient to justify a doubt that the non-transgressibility of the upper limit represents anything other than a more or less predominant tendency. In Table III. it may

TABLE V.

Reference No.	Generation I.				Generation II.		Generation III.	
	Father's Father.	Father's Mother.	Mother's Father.	Mother's Mother.	Father.	Mother.	Hair.	Eye.
1 hair. eye.	rd bl	blk blk	lt br gy	blk bl	blk dk br	br bl	1 dk br 3 rd 3 br 1 blk	dk br bl bl blk
2 hair. eye.	blk gy bl	blk br	b bl	br bl	blk br	br bl	4 br 1 br	bl br
3 hair. eye.	lt br br	blk bl	lt br gy	blk br	dk br bl	dk br gy	4 lt br 5 dk br 1 dk br	bl bl gy
4 hair. eye.	lt br bl	blk blk	blk gy	blk br	blk gy	br br	4 br 1 lt br	br gn
5 hair. eye.	— bl	br bl	— bl	blk br	br bl	rd br bl	3 lt br 1 yl	bl bl
6 hair. eye.	blk bl	— bl	— bl	br bl	blk bl gy	br bl	2 lt br 2 dk br 1 dk br	bl bl gy bl
7 hair. eye.	br bl	br br	br br	br bl	br br	br bl	2 br 2 br	br bl
8 hair. eye.	br bl	br br	br blk	br bl	br br	blk blk	1 br	bl
9 hair. eye.	br bl	br bl	br bl	blk blk	blk br	blk blk	2 br 2 blk	br br
10 hair. eye.	br bl	br br	blk blk	br bl	br br	br gy	1 br 1 blk 1 lt br	bl blk bl
11 hair. eye.	br bl	lt br bl	blk blk	lt br bl	blk bl	blk blk	1 dk br 2 blk 1 lt br 2 dk br	blk br bl gy br
12 hair. eye.	br gy	br bl	blk blk	br bl	br gy	br gy	2 br 1 lt br	bl bl
13 hair. eye.	— —	— —	lt br bl	lt br bl	br gy	lt br bl	3 lt br 2 lt br 1 br	bl gy bl
14 hair. eye.	blk bl	blk bl	au bl	blk "gn br"	blk bl	blk gn br	2 lt br 2 blk	bl bl
15 hair. eye.	blk bl	blk gy	br bl	blk bl	br bl	br bl	1 lt br	bl gy
16 hair. eye.	au gy bl	blk gy bl	blk br	br bl	blk gy bl	blk br	1 blk 1 au 2 br 1 br	br br gy bl gy
17 hair. eye.	yl bl	br br	br bl	br gy	lt br bl	br bl	1 rd	bl

TABLE V.—*Continued.*

Reference No.	Generation I.				Generation II.		Generation III.	
	Father's Father.	Father's Mother.	Mother's Father.	Mother's Mother.	Father.	Mother.	Hair.	Eye.
33 hair. eye.	br bl	blk br	lt br bl	br bl	blk gy br	sandy (rd) bl	4 br 1 br 2 lt br 1 yl 1 blk 1 lt br	gy bl bl bl br br
34 hair. eye.	blk bl	blk gy	blk bl	blk gy	rd br	blk bl	1 au 2 dk br 1 br with red streaks	bl bl br
35 hair. eye.	— —	— —	br bl	br bl	br gy	br bl	4 br 2 br 1 lt br 1 lt br	gy bl bl gy
36 hair. eye.	— —	— —	br gy	br bl	br br	br bl	2 br	bl
37 hair. eye.	— —	lt br gy	— —	— —	reddish yl bl	blk gy	1 blk 1 yl 1 lt br	gy bl bl
38 hair. eye.	blk blk	br bl	lt br bl	br bl	blk br	br bl	1 br 1 br	br bl
39 hair. eye.	blk? gy?	dk br? dk br	br gy	— —	blk dk br	lt br gy	2 dk br 1 lt br 1 lt br	dk br hz bl
40 hair. eye.	dk br bl	blk br	br bl	br br	blk gy	br br	2 br 3 br 2 blk 1 lt br	gy br br gy ga
41 hair. eye.	lt br bl	br bl	blk br	blk br	dk br bl	blk br	2 blk 1 blk 1 lt br 1 lt br	br gy gy bl
42 hair. eye.	br bl	br bl	— —	— —	br bl	blk bl	4 lt br	bl
43 hair. eye.	br bl	br bl	blk br	blk blk	br bl	blk blk	4 br 1 blk 1 blk 1 lt br	gy blk gy gy
44 hair. eye.	— —	— —	rd bl	br gy	br gy	br bl	1 br	gy
45 hair. eye.	blk bl	blk bl	— —	blk br	br bl	br bl	2 br 3 lt br	bl bl
46 hair. eye.	rd bl	blk bl	blk bl	blk blk	au bl	blk br	2 lt br 1 dk br	bl bl

TABLE V.—*Continued.*

Reference No.	Generation I.				Generation II.		Generation III.	
	Father's Father.	Father's Mother.	Mother's Father.	Mother's Mother.	Father.	Mother.	Hair.	Eye.
64 hair.	br	lt br	br	—	lt br	blk	1 blk	gy
eye.	gy	bl	—	—	bl	br		
65 hair.	br	blk	lt br	blk	blk	br	2 br	br
eye.	gy	br	bl	br	br	br		
66 hair.	blk	br	br	br	blk	br	1 dk br	bl
eye.	—	—	bl	gy	gy	gy	2 dk br	gy
							4 dk br	br
							1 lt br	br
67 hair.	—	—	—	blk	blk	blk	2 lt br	bl
eye.	—	—	—	bl	br	bl	1 blk	br
68 hair.	—	—	—	—	br	br	3 br	bl
eye.	—	—	—	—	bl	bl		
69 hair.	—	—	blk	br	blk	dk br	2 dk br	bl
eye.	bl?	bl?	bl?	gy	bl	br		
70 hair.	—	—	—	—	—	—	3 blk	bl
eye.	—	—	—	—	gy	bl	1 br	gy
							1 dk br	bl
71 hair.		blk	—	blk	blk	blk	1 blk	gy
eye.	—	br	—	blk	gy	br	1 blk	blk

be seen that black appears in 10 out of 85 cases in crosses of brown and brown ; in 8 out of 51 cases in crosses of brown and light brown, as well as in a few cases of crosses of brown with red and yellow. A few cases have come under my personal observation in which the hair of one of the offspring was distinctly darker than that of the darker parent.

It is well known that there is a certain correlation between the colors of hair and eyes, such as the association between dark hair with dark eyes, and light hair with light eyes. Table IV. shows that black hair may be associated with black eyes or with eyes containing less pigment, the combination of black and blue being not uncommon. Brown hair may be associated with brown eyes or the lighter shades, but not with black eyes. Light brown hair is associated with gray eyes or blue eyes but not with the darker colors. Auburn hair may occur with brown eyes, but red hair, which contains less melanin pigment, usually is associated with blue eyes. Probably fuller data would furnish exceptions to the above rules. According to our results dark hair

may be associated with light color of eyes but light hair is not associated with dark color of eyes. Light-haired individuals, if adults, are pretty sure to have eyes of the blue or gray type, with little melanin pigment.

The data in Table IV. show that the inheritance of the correlation of hair color and eye color is not very strong. In the first column is given the number of matings in which certain combinations occur; in the second, the number of cases among these matings in which the combination appears in the offspring; in the third, the number of cases in which the combination occurred in which it failed to be transmitted. Black eyes and black hair both behave as dominant characters, but it frequently happens that parents, one of whom has black eyes and black hair, will produce a child with black hair and blue eyes, although they will not produce one with black eyes and light hair. We cannot be dealing here with two independently mendelizing characters, because the independence is purely a one-sided one.

SUMMARY.

1. In the inheritance of the color of hair and eyes, the more pigmented condition tends to be dominant over the less pigmented.
2. Crosses of more darkly pigmented types produce a number of dark types as well as a number of lighter ones, but crosses of the lighter types rarely produce darker ones.
3. Inheritance of eye color and hair color, although partly alternative, conforms to a certain extent to the blended type.
4. There is a certain amount of evidence that the pigmentation of eyes and hair may exceed that of both parents, especially when both parents are pigmented to the same degree.
5. Dark hair may be associated either with dark eyes or light eyes, but light hair does not occur along with darker eyes.
6. Correlations of hair color and eye color are not strongly inherited.

BIOLOGICAL BULLETIN

HYDROIDS IN THE ILLINOIS RIVER.¹

FRANK SMITH.

During the past summer (1909) while at Havana, Ill., in connection with the reopening of the Biological Station of the Illinois State Laboratory of Natural History, the writer was surprised to find at one location numerous colonies of a hydroid which presumably belongs to the genus *Cordylophora*. A superficial examination of the animal and of the literature involved has not disclosed any reason why it may not prove to be *C. lacustris* Allman which is a species of hydroid commonly found in brackish water and less frequently in fresh water.

Numerous colonies were found July 30 in a partially submerged willow thicket near the north end of Quiver Lake which is really a part of the Illinois River near Havana. The majority of the colonies were attached to the submerged portions of living willow shoots while a few were found on the leaves and stems of other plants. A later visit was made to the same locality October 16 when the water of the lake was somewhat lower and no longer covered the spot at which the July collections were made. In the part of the thicket which was still submerged, numerous colonies were found attached to dead sticks and branches that projected from the bottom toward the surface. At each visit the collections were made in water less than two feet deep and over which a considerable layer of *Lemna* had accumulated under the influence of west or northwest winds. In mid-summer the colonies were in dense shade and were associated with a great variety of living organisms among which bryozoans were especially abundant.

¹ Contributions from the Zoological Laboratory, University of Illinois, under the direction of Henry B. Ward, No. 2.

In size, mode of branching, number of gonophores and of embryos in the gonophores the colonies are similar to those of *C. lacustris*, when found in strictly fresh water, as described by Pauly¹ and others. The main stems are but 1-1.5 cm. in height, but sparsely branched and the branches commonly bear but one gonophore which has usually not more than five or six embryos. Gonophores were present in July but not in October.

As opportunity has permitted, other places about Havana have been examined for hydroids, but thus far without success.

On August 4, during a brief visit to the Illinois River bottomlands and lakes near Hennepin, I noticed on a partially submerged concrete wall extensive areas bearing organisms that seemed to be hydroid colonies similar to those found at Havana. As there was no time nor equipment for examination of the living material nor for its proper preservation, there is nothing at hand to serve as a basis for the identification of this form except the macerated remains of a few colonies which were scraped off and kept in a vial of water. The skeletal remains of the colonies are indistinguishable from those of the Havana species and I feel quite sure that the forms are identical and that the species is well established at two places in the Illinois River nearly a hundred miles apart.

How long this hydroid has been represented in the Illinois River is problematical, but to the writer it seems somewhat improbable that it was in the Havana region before the opening of the Chicago Drainage Canal, as for several years prior to 1900 various observers connected with the Biological Station searching persistently for all kinds of animal life found no such hydroid forms. There is at least a possibility that this hydroid may be found about the docks in the Chicago region where it may have been introduced by vessels from the Atlantic coast and then subsequently have been carried through the drainage canal into the Illinois River. More extended observations on its distribution in the Mississippi Valley are highly desirable.

UNIVERSITY OF ILLINOIS,
November, 13, 1909.

¹ *Zoologische Anzeiger*, Vol. XXIII., pp. 546-551.

THE ANURA OF ITHACA, N. Y.: A KEY TO THEIR EGGS.

ALBERT HAZEN WRIGHT.

For the last four years the writer has been studying the life-histories of the Anura of Ithaca, N. Y., but it will be some time before the work reaches completion. It seems best, however, to present the following brief summary of one phase of the work, in the hope that it may be of help to the numerous workers who employ Anuran eggs either for comparative or experimental embryologic purposes.

Eight species of Anura are found at Ithaca, N. Y., namely: *Rana sylvatica*, *Hyla pickeringii*, *Rana pipiens*, *Bufo lentiginosus americanus*, *Rana palustris*, *Hyla versicolor*, *Rana clamitans* and *Rana catesbeiana*.

The first five species appear from hibernation and spawn under a maximum air temperature of 43°–50° F.; the last three delay until the maximum reaches 70° F. or more. The first five usually breed from the last of March until the middle of June; the last three, from the last of May into August. All but two species, *Bufo l. americanus* and *Rana clamitans*, occupy four or five weeks for the spawning period. The exceptions may require two or three months. The number of eggs in a complement varies from 800 in *Hyla pickeringii* to 20,000 in *Rana catesbeiana*.

The eggs of three species, *Hyla versicolor*, *Rana clamitans* and *Rana catesbeiana*, float more or less at the surface of the water; the eggs of the other five are submerged. The five species with submerged eggs are first to breed. They deposit eggs with firm jelly envelopes, several eggs appearing at an emission except in *Hyla pickeringii*, where only one appears at an emission. The three with buoyant eggs breed after May 25. They deposit at the surface masses or films of eggs with loose jelly envelopes, several eggs being deposited at an emission.

At the outset the attempt to secure fruitful mating with captive specimens was abandoned. The effort was rather to obtain pairs

already mated in nature. These were usually captured on night trips and were immediately taken to the laboratory. By the next morning an egg complement was ordinarily recorded. In this way a check was established upon the identification of eggs deposited in nature.

The measurements are based on fresh eggs none of which is beyond the fine morula stage. The color characters of the vitellus were taken at the time of oviposition with 7 species and not later than 6 or 8 hours after oviposition with the other species. A summary of the egg characters of each species follows in the accompanying key:

KEY TO THE EGGS OF ITHACA ANURA.

- A. A single row of eggs within a long spiral string of jelly looped about plant stems, sticks or resting upon the bottom; vitellus diameter 1.0-1.4 mm.; inner envelope diameter 1.6-2.0 mm.; outer envelope diameter 3.4-4.0 mm. Egg complement, 4,000-7,000. Season at Ithaca, April 20-July.....*Bufo lentiginos americanus*.
- AA. Deposited singly or in a mass.
 - B. Deposited in a firm consistent mass enveloping grass stems, twigs, etc., or free; submerged; often 15-20 bunches within an area of a few square feet.
 - C. Small distinct inner envelope evident to the naked eye, 2.3-3.0 mm.
 - D. Vegetative pole yellow; animal pole brown; mass globular; vitellus 1.6-1.9 mm.; outer envelope, 3.6-5.0 mm. Egg complement, 2,000-3,000. Season, April 25-May 15.....*Rana palustris*.
 - DD. Vegetative pole white; animal pole black; mass plinth-like; vitellus 1.6-1.8 mm.; outer envelope 4.2-6.0 mm. Egg complement, 3,500-4,500. Season, April 10-May 15.....*Rana pipiens*.
 - CC. Large inner envelope apparently absent, evident only under the lens, 3.6-5.8 mm.; vitellus 1.8-2.4 mm.; outer envelope 5.2-9.4 mm.; mass globular; vegetative pole white; animal pole black. Egg complement, 2,000-3,000. Season, April 1-30.....*Rana lythtica*.
 - BB. Deposited not in a hard consistent mass.
 - C. In small bunches or attached singly; vitellus, .9-1.2 mm.
 - D. Outer envelope loose, 4.0-7.8 mm.
 - E. Inner envelope, 1.6-2.0 mm.; vegetative pole yellowish; in small bunches (4-25) usually floating at the surface of the water, either attached to vegetation or free; outer envelope, 4.0-6.0 mm.; vitellus, 1.1-1.2 mm. Egg complement, 1,500-2,000. Season, May 20-July 1.....*Hyla versicolor*.
 - EE. No inner envelope; vegetative pole white; in bunches (20-100) usually attached beneath the surface of the water; outer envelope usually 5.0-7.8 mm., rarely 3.0 mm.; vitellus .9-1.2 mm. Egg complement, 500-800. Season, March 20-April 15
Chorophylus triseriatus.¹
 - DD. Outer envelope firm, 1.4-2.0 mm.; vegetative pole never yellow;

¹ Introduced at Ithaca in April, 1909.

- single or in small bunches (4-12) attached to grass beneath the surface of the water; vitellus, .9-1.1 mm. Egg complement, 800-1,000. Season, April 5-May 10.....*Hyla pickeringii*.
- CC. In large loose masses; vegetative pole white; animal pole black; vitellus, 1.2-1.7 mm.; at or near the surface of the water.
- D. Usually one continuous film, 1-2 eggs thick, on the surface of the water, the film's diameter being seldom 1 foot; inner envelope distinct, 2.8-4.0 mm.; egg mass usually attached or amongst vegetation. Egg complement, 3,500-4,500. Season, May 25-August 10.
Rana clamitans.
- DD. Either a film 1-2 1/2 feet in diameter or a stringy frayed widespread mass; attached to twigs or sticks; almost invariably amongst brush and at or near the surface of the water; no inner envelope. Egg complement, 12,000-20,000. Season, June 20-July 25.
Rana catesbeiana.

A NOTE ON REDUCTION IN THE MATURATION OF MALE EGGS IN APHIS.

N. M. STEVENS.

In discussing the unpaired heterochromosome in aphids, in 1908,¹ I expressed the opinion that the two heterochromosomes of the parthenogenetic generations must pair before maturation of the male-producing eggs, and separate in the maturation mitosis, one undivided heterochromosome going into the polar body while its mate remains in the egg. The only evidence which I could give in favor of this surmise was two equatorial plates where seven chromosomes appeared in the maturation of parthenogenetic eggs, instead of the eight chromosomes characteristic of the species (Plate II., Figs. 52 and 53). The largest of the seven chromosomes was evidently equal in volume to the sum of the two largest in the plate containing eight. I have never found the males of this species, the parthenogenetic generations continuing up to the time when the host plants are killed by frost. The probability is that a few scattered sexual forms occur among the parthenogenetic, and that the eggs with seven chromosomes were male-producing eggs.

In many species of aphids the same individual may produce both males and females and often parthenogenetic offspring also, making it very difficult to be certain that one has the male eggs. In the dimorphic red and green aphid found on *Enothera biennis*, however, the rule is, that in October parthenogenetic young cease to appear; apterous mothers produce only male, and winged mothers only female offspring. Only two exceptions to this rule have been observed. Two years ago I had one brood of males produced by a winged mother, and recently, in examining sections of an apterous specimen, I found four large parthenogenetic embryos, while all of the smaller embryos were male. The change here from parthenogenetic to sexual reproduction came during a generation instead of between generations.

¹ "An Unpaired Heterochromosome in the Aphids," *Journ. Exp. Zool.*, Vol. VI., No. 1, Jan., 1909.

This year an unusual number of the apterous, male-producing individuals were secured. Some were fixed and sectioned, others dissected and the eggs and embryos studied in Schneider's aceto-carmine. The sections gave no favorable stages. Out of a large

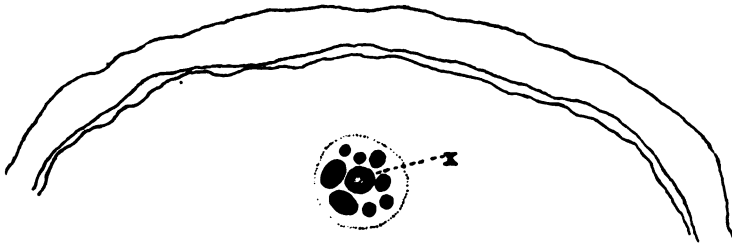


FIG. 1. Equatorial plate of maturation mitosis of male aphid egg. Outline of egg and membrane shown above. *x*, the double chromosome. Zeiss 1.5-6, cam.

number of aceto-carmine preparations, one egg was found which had the maturation spindle in metaphase. This was taken from an individual in which the older embryos were certainly male. The equatorial plate contained nine chromosomes, ten being the

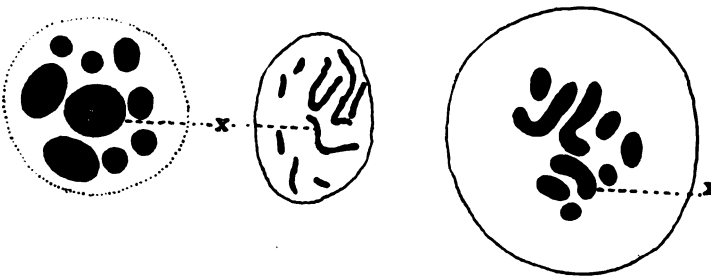


FIG. 2. Same equatorial plate drawn with Zeiss 1.5-12.

FIG. 3. Nucleus of embryonic cell (δ) in prophase. *x*, the unpaired heterochromosome. Zeiss 1.5-6.

FIG. 4. Male embryonic cell in metaphase. *x*, the unpaired heterochromosome. Zeiss 1.5-12.

number in non-sexual parthenogenetic eggs ('05, Pl. I., Figs. 7 and 12¹). This metaphase is shown in Figs. 1 and 2. The chromosome in the center of the group is the double one. In the first spermatocyte the lagging heterochromosome is the

¹ "A Study of the Germ Cells of *Aphis rosa* and *Aphis anthera*," *Journ. Exp. Zool.*, Vol. II., No. 3, Aug., 1905.

second in size ('09, Pl. II., Figs. 54, 56, 57¹) and here it is evidently the two second in size, which have fused to form the large vacuolated chromosome *x*. No prophases or anaphases were found.

In the young male embryos many cells were in mitosis and in a few cases it was possible to count and draw the chromosomes. Fig. 3 is a nucleus in prophase, flattened so that the nine chromosomes are nearly in the same plane. It will be seen that the two longest form a pair, while the next in size is unpaired. Fig. 4 is a metaphase from another embryo. The unpaired chromosome is again the second in size.

This evidence, so far as it goes, indicates that one whole chromosome goes into the polar body of the male egg, leaving

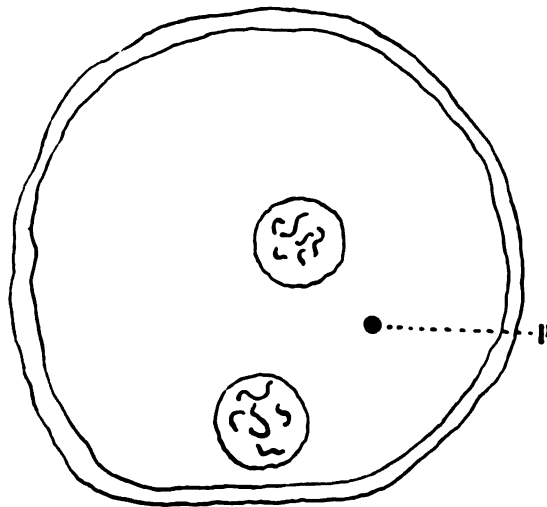


FIG. 5. Male egg showing the single polar body (*p*) and two nuclei. Zeiss 1.5-2.

the somatic number for the male individual reduced by one. Ordinarily one would not lay much stress on the conditions observed in a single egg, but considering the difficulties involved in securing favorable stages of maturation in male eggs of aphids, and the fact that the observations are in accord with Morgan's²

¹ "An Unpaired Heterochromosome in the Aphids," *Journ. Exp. Zool.*, Vol. VI., No. 1, Jan., 1909.

² "A Biological and Cytological Study of Sex Determination in Phylloxerans and Aphids," *Journ. Exp. Zool.*, Vol. VII., No. 2, Sept., 1909.

observations on male eggs of Phylloxera, it seems desirable to bring the results obtained from this autumn's collections of the *Enothera* aphid, to the notice of those interested in the subject.

In a recent paper, entitled "Mendelian Inheritance of Sex," Hagedoorn has quoted me as authority for a statement that male aphid eggs give off two polar bodies. I have found only one polar body in male and other parthenogenetic eggs, and have so stated the fact with all the evidence at hand ('05, Pl. II., Figs. 17 and 18, and text p. 317). Fig. 5 shows one of many early segmentation stages of male eggs found in aceto-carminc preparations, showing only one polar body

BIOLOGICAL LABORATORY,
BRYN MAWR COLLEGE,
November 25, 1909

A METHOD TO TEST THE HYPOTHESIS OF SELECTIVE FERTILIZATION.

T. H. MORGAN, F. PAYNE AND ETHEL N. BROWNE.

The hypothesis of selective fertilization has played an important rôle in several recent theories of sex determination and to some extent also in modern Mendelian speculations. It has been assumed for example that there are two classes of spermatozoa and two classes of eggs—male and female eggs and male and female sperm—and that fertilization is reciprocal in the sense that a male sperm can only fertilize a female egg and that a female sperm can only fertilize a male egg. On the other hand the more commonly accepted view is that any sperm can fertilize any egg.

Until this question is settled by direct observation or by experiment these two alternatives will continue to make uncertain our interpretations.

To put together one sperm and one egg would seem to be the simplest way to test the question. The operation may not in itself present insuperable difficulties but the chance of the spermatozoon reaching the surface of the egg is so small as to make the attempt rather hopeless; for there is no evidence to show that the spermatozoon is attracted towards the egg. The work of Buller, in particular, shows that accident alone determines the contact between the spermatozoa and the membranes, or the jelly, of the egg.

By means of the following simple method we have found it possible to study the problem of selective fertilization. We feel that while the number of cases here recorded is too small to settle so important a question, its application on a larger scale and on other animals should furnish conclusive evidence for or against selective fertilization.

We wish therefore at present to lay more emphasis on the possibilities of the method than on the certainty of demonstration from the number of recorded cases, and hope that others

may be led to study this important question by the same method.

We made use of the eggs and sperm of the mollusk *Cumingia*. The spermatozoa are quite large and can be readily seen with moderate magnification. The eggs are small so that the entire exposed surface can be watched closely. The eggs just laid were put into a drop of water on a slide, and a cover slip added. The cover slip was sufficiently supported so that the eggs were not too much compressed. It was advantageous to apply pressure because otherwise the spermatozoa may reach the egg above or below the horizon of observation; such sperm are lost to sight as a rule and their fate remains uncertain. Even with moderate pressure the spermatozoön sometimes slips in between the egg and the cover slip (above or below the horizon) and are lost to sight. In such cases further observation is worthless.

By means of a fine pointed pipette a small drop of water containing not too many spermatozoa was introduced at the edge of or under the cover slip. From the point of insertion the spermatozoa swim out in all directions and at some distance from the starting point the path of a single spermatozoön could be easily followed. The only way in which we were absolutely certain of seeing the first spermatozoön that reached the egg was to focus on an egg and wait until one came in contact with the egg.

It was seen that many spermatozoa swim past the egg and show no evidence of turning towards it, but those whose previous path was such that they ran into the jelly around the egg, bored into the jelly and often reached the surface of the egg. Whether after a spermatozoön has entered the jelly it ever turns towards the egg (if it did not have this orientation at the time of contact) is difficult to determine with certainty, but it is certain that spermatozoa may bore through without turning towards the egg. Some of the attached spermatozoa may show alternate periods of rest and activity, and in consequence change their position several times, and even end by entering the egg, but there is no evidence that one position is more directed than another.

The successful spermatozoa are those that strike the egg "head-on," and bore directly towards the surface. When the surface is reached the end of the spermatozoön appears to enter

the outermost layer of the egg. As a rule a pause follows, and it may take the spermatozoön from five to twenty minutes to disappear within the egg. The penetration is, as a rule, at first slow but later the spermatozoön may enter quite suddenly. Whether the sperm bores its way into the egg, or whether there is first a reaction between the surface of the egg and the spermatozoön so that the egg also takes a part in the process need not be discussed here, but it is important to note that no spermatozoa enter except those that stand with their long axis vertical to the surface and pierce the surface with the tip of the sperm head.

Our observations show that the first sperm that fulfills these conditions is received. In some of these cases a second sperm came in contact with the egg after the first had come in contact with it. In all such cases the first sperm only penetrated. There was no evidence in favor of selective fertilization, since in all forty cases the first sperm that approached in the normal position was the first to enter. It is highly improbable that forty times, this first sperm was the one suited to enter (assuming that two kinds exist) when there is no evidence that the eggs attract the sperm. Our general conclusion from the data here presented is that in this case selective fertilization does not occur, and since *Cumingia* is unisexual the temptation is to generalize this statement to include all such forms. Whether this extension is warrantable or not the fact remains that in this, the only case so far tested, the evidence is opposed to the hypothesis of selective fertilization.

COLUMBIA UNIVERSITY,
November 20, 1909.

THE ANATOMY OF THE STYLETS OF CAMBARUS AND OF ASTACUS.

E. A. ANDREWS.

In all the crayfishes, *Cambarus* and *Astacus*, of the northern hemisphere, the limbs of the first and second segments of the abdomen of the male are modified in a peculiar way and are used as instruments to transfer the sperm from the deferent ducts to the outside of the body of the female. These limbs we will call the first and second pairs of stylets.

In *Cambarus* it was found (1, 2) that the stylets place the sperm within a special sperm receptacle in the shell of the female. In *Astacus*, as far as is known, the stylets deposit the sperm over the shell of the female in secreted tubules, or spermatophores.

While the stylets are much alike in the two genera the following study shows that the parts directly concerned in the passage of sperm present two stages of perfection, those of *Cambarus* being the more specialized. The first pair of stylets are firm, awl-like structures, which in *Astacus* are evidently comparable to a rolled in plate, while in *Cambarus* they seem solid with only a superficial groove. But we now find that this groove is the outlet of a concealed tubule and that in *Cambarus* also the organ may be regarded as a modified plate.

As the second stylets are much alike in the two genera and have a subordinate rôle to play in the process of sperm transfer we will consider chiefly the first pair.

It is a remarkable fact that the first stylets in these crayfishes present specific differences so that the systematist relies upon the forms of the stylets as an important aid in the description and identification of species. The figures of Hagen (3) and of Faxon (4) show the great amount of diversity in proportion and in character of termination in many species of *Cambarus*. But despite this diversity in form the following description of the first stylets in four species taken at random and representing four of the six subgenera of *Cambarus* makes it probable that in all species the stylets have the same essential anatomy and use.

We will first describe the stylets of *Cambarus* and then those of *Astacus*.

In these reduced limbs (Figs. 1 and 2) we may distinguish the base (*B*), the neck (*N*) and the spiral (*S*) which is the region with a somewhat spiral lengthwise groove bounded by hard rounded edges that run out to form the two tips of the whole organ. It is this bifid appearance of the limb which has been most emphasized in descriptive work. One of the tips may in this species be called the spatula (*Sp*) from its shape. The other tip (*C*) may be called the canula, as it is a termination of a tube and is inserted into the sperm pocket of the female and allows the sperm to pass out of its tip. Of the two apparent tips of the limb, one, the spatula, is thus a side outgrowth of minor importance; the other is the real morphological and physiological end of the organ and of fundamental value.

The groove that runs along the length of the spiral region begins at an orifice (*Or*) and ends at the extreme tip of the canula.

We may regard this groove as dividing the spiral region into two parallel portions, the external mass (*Ex.m.*) and the median mass (*M.m.*), external and median being used with reference to the median plane of the entire animal.

In *Cambarus virilis* (Figs. 1 and 2) the stylet is exceptional in the great elongation of the spiral region, the spatula being very much prolonged and the canula a curved, ovipositor-like structure. The tufts of setæ at the junction of neck and base and upon the median mass near the orifice are also long.

Cross-sections (Fig. 3), at the level 3 of Fig. 2, show that the external groove of the canula passes deep into the interior and has its inner end partly cut off as a tubule by a ridge or shelf which projects like a valve from the side of the groove. Serial sections show the same general facts throughout the length of the spiral. There is thus a continuous tubule from the orifice to the tip of the canula.

While the stylet has most of its exoskeleton firmly calcified, as represented by the black in the section, the tips of the spatula and canula are partly horn-like. In the median mass this horn extends some distance toward the base, as represented in the dotted area in the figure. The shelf that overhangs the tubule

is also of horny and not of calcified material and thus may the better make a closure of the tubule.

The living tissue of the stylet was found to be a loose areolar mass full of blood spaces and covered by a thin epidermis that makes the exoskeleton. This tissue filled the vacant space in the external mass in Fig. 3.

Excepting the muscles in the base that move the whole limb upon the body there are no muscles within the stylet, but on the other hand there are largely developed glands in the swollen proximal parts of the spiral. These glands discharge through the shell into the tubule not far from the orifice.

In *Cambarus diogenes*, which belongs to the subgenus *Bartonius*, the stylets (Figs. 4 and 6) have a very different appearance owing to great shortness of the terminal portion of the spiral. The base and the neck remain much as in *C. virilis*, but the spatula and the canula are very short and thick with the tips turned up dorsally (Fig. 4). They are also much flattened, and are thus very narrow as seen from the posterior face (Fig. 6).

Practically the whole length of the canula and much of the spatula are horny. A section across the canula (Fig. 5) shows the shelf from the external mass (*Ex.m.*) and the isolated bottom of the groove. It also shows that the median mass (*M.m.*) has exaggerated the tendency seen in *C. virilis* (Fig. 3) to grow over the groove, to such an extent that it runs over the shelf of the external mass and so makes the closure of the tubule a very complete one.

The horny tip of the canula shows its finer structure more readily than in *C. affinis* and we see under higher magnifications that the horny substance presents lengthwise striations on the surface, which at the tip give place to areolations suggesting scales. Possibly this slight roughening of the tip of the canula may be of some use in cleaning out the orifice of the sperm receptacle.

In the southern form, *Cambarus Clarkii*, the first stylet is the antithesis of that of *C. virilis* for the terminal parts of the spiral (Fig. 7) are so greatly shortened as to form a flat mass that is largely horny and though bent upward, somewhat as in *C. diogenes*, more complicated at its tip.

The setæ are short but form very long rows. Those of the base extend along the neck far up onto the external mass and those of the median face run nearly its whole length. There are also some additional setæ upon the dorsal face near the tip as seen in Fig. 8.

In following the groove (Fig. 7), we find that after it passes the very slender and insignificant spatula it turns ventrally to end on a blunt protuberance indicated in the dotted area in Fig. 7. There is left a large protruding mass external to the spatula, which is indicated in parallel shading, and which is evidently part of the median mass that does not extend to the very tip of the groove. Thus the tip of the stylet is bifid; the blunt part to the right in the figure is the canula and the part to the left is a sharp blade formed from the median mass beyond the spatula and this we will call the scalpel, from its shape and its probable use in opening the very firmly closed and constricted slit of the annulus in the females of this species. The scalpel is apparently well placed to cut open the orifice of the annulus (see 1, Fig. 24).

The character of this condensed terminal region is better seen in the enlarged view of the external face of the tip (Fig. 8). The scalpel, to the right, bears the above-mentioned setæ at its base and has a sharp convex edge.

It is set off from the canula by a depression. The canula itself, as seen behind the outer parts of the setæ, is slightly bifid in this preparation but in dried specimens it ends with a terminal orifice. While the stylet has two elastic tips the canula (*C*) and the scalpel (*Sc*) they are so near together that both might readily enter the orifice of the annulus.

On cutting sections of this unusual stylet we find that the groove has its bottom cut off by a shelf (Fig. 9), just as in the other species. But the tubule so formed is so minute and so deeply buried that it is easily overlooked. A section (Fig. 9) taken near the base of the spatula at ρ (Fig. 7) shows the median mass so extended as to make a sharp blade, the scalpel (Fig. 8). And moreover the shelf that arises from the external mass, at this level, points away from the median mass, so that the section is not readily compared with that of *C. virilis* (Fig. 13). In sections the horny shelf can be traced down from the free edge of

the end of the canula and represents the edge of the external mass along the side of the groove, but before the orifice at the base of the spiral is reached the shelf disappears and does not continue on as part of the lip of the orifice.

Within the minute tubule a small number of sperms were seen.

The use of these organs in the processes of sperm transfer was seen to be the same as in *C. affinis*. In brief the phenomena were: The hooks of the third legs hold the male firmly to the female. The male holds the claws of the female. The first and second stylets are locked together and the fifth leg is crossed. The pleopods are swung back and forth. The second stylet glides a millimeter or so up and down the first, with its wedge in the groove. Occasionally jerks of the base of the abdomen make slight hammering thrusts of the tip of the stylet-complex. While both stylets present their tips to the slit of the annulus it seems difficult for both to enter at once since the slit is median and not transverse. The exopodite of the second stylet shows some slight twitching movements. The female pleopods of the first somite extend over the annulus and touch the setose palp of the stylet. On removing such a conjugating male and placing the fifth leg across to support the locked stylets, sperm issued from the hole at the tip of the canula and in a few minutes sperm came out of the tips of both papillæ. In separating a pair one stylet was very firmly fastened in the annulus and tended to pull the annulus away with it. This stylet had shoved the annulus up into the body of the female as far as possible. This attached stylet was on the same side as the crossed fifth leg.

Cambarus Montezumæ is a representative of the subgenus *Cambarellus* from Mexico, and should present, in some respects at least, a more nearly ancestral state than the above species.

Nothing is known of the conjugation habits, as the species is known only from preserved specimens. The male has two hooks on each side, and presumably both are used as is the one in *C. diogenes*. These hooks (Fig. 10) are on the second and the third legs and are like those of *C. affinis* but less blunt. Those of the third legs are the longer, sharper and more specialized in form.

The stylet (Fig. 11) is short and simple, with the usual tuft of setæ absent from the median face which is very wide and flat.

The spatula (*Sp*) is very large and hollowed on the median face to form a wide spoon. The canula (*C*) is sharp-pointed and somewhat curved, with a horny tip that plainly shows the groove running to it and opening by a hole. There is also a new outgrowth (*L*) that has the form of the spatula of *C. virilis* but arises from the external mass, half way between the origin of the spatula and the tip of the canula. This new outgrowth we will call the ligula.

Cross-sections of this stylet (Fig. 12 at the level 12 of Fig. 11) show the presence of a shelf that cuts off the bottom of the groove as a tubule, similar to that in *C. virilis*. This shelf has the same yellow, homogeneous appearance. This section shows the long flat extension of the median mass that forms the base of the spatula and the shelf at the bottom of the overhung groove.

In the section (Fig. 13 at the level 13 of Fig. 11) we see the orifice and in the median mass (*M.m.*) glands with one of the tubules discharging through the exoskeleton into the groove.

This specimen seems to have been about to shed, so that the exoskeleton is represented rather schematically in the sections, as it was broken or laminated.

Turning now to the genus *Astacus*, to which all the European crayfish belong, the process of sperm transfer is known only from the brief accounts of Soubeiran (5), Chantry (6) and Huxley (7). From them it appears that the males seize and turn the females and mount them, but the subsequent stages differ from those in *Cambarus* in the fact that there are neither hooks nor annulus, and thus no transfer of sperm to any sperm pocket; on the contrary, the sperm passes out of the stylets onto the sternal surface of the female in the form of spermatophores. These subsequently liberate the sperm at the time of laying in some unknown manner that Whitman (8) states was referred by Leuckart to the compression of the walls of the spermatophores and by Meyer to the action of the secretion that fastens the eggs. That the same general method is followed by the *Astacus* of the west coast of the United States seems undoubted from the similarity in the anatomy of the organs concerned and from the following observations.

Amongst female crayfish of the species *A. leniusculus*, received

from Oregon in February, or in October, 1904, there were a few that presented the remnants of spermatophores on the ventral surface of the thorax. In the best marked case these were some hundred empty tubes, 3 to 4 mm. long, and more than .25 mm. thick, of red-brown color, stuck close to the shell of the female for the most part, though some had one free end standing up about a millimeter into the water. Most were laid down carefully side by side in groups. Some few were twice the usual length. A few were on the base of the second leg, on one side; more were at the base of the third leg and close to the opening of the oviduct. Still more were at the bases of the fourth legs and between them just anterior to the annular plate, onto which two spermatophores extended. Two were on the sternum between the fifth legs. The entire collection, in a sketch, forced one's attention to the fact that they had either been originally placed in depressions and angles where they would not be readily rubbed off, or else that these seen were the survivors that had escaped removal after more unprotected spermatophores had gone. Each spermatophore had its tips greatly contracted, as if a soft material had shrunk more at the end, somewhat like egg-cocoons of earth-worms.

Thus the male of the American crayfish *Astacus* must deposit the sperm in tubes over the ventral side of the thorax of the female, and not introduce it into any special cavity.

In comparing the sperm-transfer apparatus here with that of *Cambarus* we find greater simplicity, as was to be expected for the performance of this less specialized mode of transfer.

The first stylet of this *A. leniusculus* (Fig. 14) is like that of the English *Astacus*, as figured by Huxley (7) in the main, while also being like the stylet of *Cambarus*. The base is simple and without the specialization of form to nicely accommodate the second stylet, but the ridge along the neck bears setæ. The spiral is obviously a hollow cone or tapering scroll with a very wide orifice between the long external, and the shorter median mass. The groove is much more open than in *Cambarus* and the whole organ is less rigid and seems as if well made for a mere conduit and not for an organ to be forced into a hard slit. The stylet is not noticeably bifid and the rather blunt tip is the canula

while the spatula and all other lateral outgrowths are absent. Huxley says of *A. fluvialis*, "terminal half of the appendage is really a broad plate, slightly bifid at the summit, but the sides of the plate are rolled in in such a manner that the anterior half bends around and partly encloses the posterior half. They thus give rise to a canal, which is open at each end, and only partly closed behind." In *A. leniusculus* this overlapping of the one part by the other has proceeded much farther, so that in Fig. 14 we see the anterior, or median, mass has covered in and concealed the external mass through all the terminal extent.

In sections this extensive inwrapping becomes at once patent. The section 15 shows a widely open canal closed in not only by the rolled median mass but internally by an opposite rolling of the external mass; that is, the hypothetical plate, of which the terminal part of the stylet is composed, has both its edges rolled in, first, the external edge and then the median edge outside the other. Both flaps come so close together that near the tip of the canula 15 (Fig. 14) the central tube is well shut off from the water (Fig. 15). Contrasting this with *Cambarus* (Fig. 3) we see that in both cases there is a horny plate inrolled, but in *Astacus*, this is much like the rolling up of a sheet of paper, while in *Cambarus* it is the buckling up of a thickened mass whose edges meet over a groove. *Cambarus* shows the derived, the more special, the less mechanically direct sort of inrolling.

Farther down the stylet (at 16, 17, 18, of Fig. 14) the rolling is more and more imperfect (Figs. 16, 17, 18). These figures show the rather thick calcified shell and the usual connective tissue, but the absence of glands is conspicuous. Also the edge of the external mass becomes specialized as a sharp shelf that overarches the large central canal, while the enveloping median mass still overlaps the external mass and makes the closure of the canal a more firm one.

In Fig. 17 there is a complexity of the canal that exists for a short distance and may prove to be of some significance when the process of sperm transfer can be studied. There is a narrow side-slit from the groove, to the right in the figure, which is made by special thickening of the shell of the external mass. That is, there is a ridge along the bottom of the groove. With this ex-

ception, there is nothing to suggest the minute inner part of the groove of *Cambarus* and the large hole covered by the shelf must be the homologue of the tubule of *Cambarus*.

The obvious suggestion that the canula is derived from a rolled plate is unfortunately supported by no actual observation, though the few following facts regarding the development of the stylet in *A. leniusculus* show a simple beginning that may well later suffer a process of inrolling.

A larva 19.5 mm. long, shedding from the fourth to the fifth stage, showed on the cast the two minute papillæ seen in Fig. 19, growing toward one another on the ventral ridge of the sternum of the first abdominal somite. That these are the first stylets of the male is indicated by the fact that in larvæ killed in the middle of July and presumably in the fourth and fifth stages nine showed no outgrowths and were probably females while six showed outgrowths similar to these in Fig. 19. These little stylets differed much in the different males.

In larvæ from 20 to 26 mm. long the stylets differed in size and form from the state shown in Fig. 19 to that shown in Fig. 23. The state of advance of the stylet was not parallel to the length of the larvæ, thus a male 26 mm. long had the stylet much as in Fig. 19, while one 25 mm. long had them as Fig. 23. A male 20 mm. long had the stylets shown in Fig. 20; they were somewhat flattened papillæ pointing toward one another. Fig. 21 shows the left stylet from a male 23 mm. long and Fig. 22 that of a similar male. The most developed stylet (Fig. 23) is not only flattened but its posterior face is somewhat concave and shows on its median edge, to the left in the figure, a slight notch to represent the future neck (compare Fig. 14), while the median edge is thickened as if it might grow up to form the enveloping median mass to cover over the flattened or concave part that would be the groove. The whole organ is then a stiff flat spoon and is remarkably like the stylet of the American lobster in miniature.

At the tip of the stylet is a minute protuberance tipped with a spine and suggesting a sensory function (Fig. 23). This was found on the stylet of the opposite side, but not in any of the stylets of other males, which were all less advanced.¹

¹ The general proportions of the longest 26 mm. male may be seen from the following measurements: The length of antennæ 26 mm., the chelæ 18 mm., the width

While the first stylets of both *Cambarus* and *Astacus* show neither in their anatomy nor in their ontogeny any sign of derivation from the typical forked crustacean limb this is not the case with the second stylets. These remain forked in the adult and we shall see that they arise from the modification of an ordinary pleopod by the addition of a lateral outgrowth, while the first stylet would seem to be derived by the dropping out of part of the typical limb and by the condensation of the rest.

The second stylet in *Cambarus* bears a setose exopodite and endopodite that are fringed with setæ and contain muscles that move these forks of the limb upon the basal part. In the development of the larva there is added to the simple limb an outgrowth from the endopodite that finally becomes the peculiar excrescence characteristic of the second stylets of the crayfish. These outgrowths are applied by the adult male against the orifice of the first stylet and play an important accessory rôle in the processes of sperm transfer.

In *Astacus* the second stylet (Fig. 24) is much like that in *Cambarus*. It has a slender exopodite (*Ex*) and a wide endopodite (*En*) that ends in a setose filament (*Fl*). But the lateral outgrowth, or excrescence, is different. In *Cambarus* this part may be called the triangle and it ends in a rounded free edge that is inserted into the groove of the spiral of the first stylet during sperm transfer. This thick edge is somewhat comparable to a radius bone and ends with a hollowed head. Distal to this radius the triangle is continued as a pyramid, bearing setæ: this pyramid we call the "wedge."

But in *Astacus* (Fig. 24), the triangle (*Tr*) suggests an extinguisher in form since the wedge (*W*) is a direct continuation of the edge of the head of the radius in the form of a curved plate as indicated in the smaller figure to the right. The wedge (*W'*) is not a pyramid at all but a thin, rather pointed plate curved around a deep depression to join the edge of the radius (*R*) as shown in Fig. 24.

of thorax 8 mm. (in alcohol), of telson fan 11 mm., of telson 3.5 mm., of setæ on telson 15 mm., number of joints in antennæ 75 plus. The stylets were about 2 mm. and the second stylets 4 mm. apart. There was a rounded area and a dim white organ within base of fifth leg, indicating the deferent ducts of the male, probably.

From the state found in this *Astacus*, the more "extinguisher-" like shape found in the English *Astacus* could be formed by a process of simplification, or reversal of specialization, just as is true for the first stylets. The above mentioned larvæ of *Astacus leniusculus* furnished but meager facts bearing upon the ontogeny of the second stylet, but this is enough to establish the existence of an early modification of the median edge of the endopodite to subsequently form the triangle or scroll. The second pleopod is at first like the following ones and only gradually takes on the specializations that make it an accessory sperm-transfer organ. The earliest detected modification of the endopodite was a slight groove followed in larger larvæ by an elevation on which the same groove was seen. How this groove on an elevation gives rise to the triangle remains for study of later stages to decide.

In Fig. 27, which is the anterior face of part of the second pleopod of a male, there is a marked protuberance on the median side of the endopodite, and this contains a lateral groove. In some other males the groove was present, but not the elevation. Thus Figs. 25, 26 represent the anterior and the posterior faces of the edge of the endopodite of a male 23 mm. long, showing only the exoskeleton and the plumose setæ. The groove is a transverse pit which ends abruptly on the anterior face of the endopodite; it is bounded distally by a slight lip-like transverse ridge standing out into the water.

There is thus a transverse pit on the median face of the endopodite at the region that will later be part of the triangle (compare Figs. 27, 24). The cells of the epidermis were small and ran in as a single layer to line the pit and extend into the lip as a solid mass.

In the more advanced stage Figs. 27, 28 this same pit is on a decided elevation. The pit is a transverse slit still lined with epidermal cells (Fig. 28), but its distal edge is no longer a lip but only part of the general elevation. This male was the one having the advanced first stylets seen in Fig. 23. The posterior view (Fig. 28) is intended to show the epidermal cells in surface view as well as in optical section and also the fact that the pit opens gradually onto the general level on this posterior face, while on the anterior face it ends abruptly at a steep wall lengthwise of the endopodite.

No doubt the general elevation later becomes the triangle and is comparable to the knob found in *Cambarus*, but the meaning of the lateral pit is problematical. Possibly it is the forerunner of the cavity at the end of the triangle which gives it the extinguisher-like form, and which being still more prominent in the English *Astacus* may be an old trait that would more likely find expression in the older genus, *Astacus*, than in the newer one *Cambarus*.

By way of summary we will state that the first stylet of these crayfishes, *Cambarus* and *Astacus*, has the anatomy of a pleopod that has lost both its biramous form and its intrinsic muscles and has become a nearly closed tube. In its physiology it is essentially a tube to transmit the sperm from the male to the female.

The ontogeny throws little light upon the phylogeny of the organ since at its first appearance in the larva it is already a simple papilla, which in *Astacus* becomes a flat plate that then rolls in to form a tube while in *Cambarus* it forms a tube by thickening of the edges.

The apparent simplicity of the first stylet in *Cambarus* misled Hagen (3, p. 17) to regard it as having lost its channel save for the external groove, while in reality there is a functional inner tubule.

The anatomy of the first stylet of *Cambarus* gives a firm basis for the interpretation of the various terminations of this organ as exhibited in different species and made use of for detecting genetic relationship as well as specific characters. It will be necessary to restudy the stylets of all Cambari to determine in how far the accepted morphological division into "inner" and "outer" parts is a sound basis for comparisons. In each species the canula, or real termination of the organ, must be distinguished, and the various secondary outgrowths classified as to their origin from the two sides of the groove that ends at the tip of the canula. With this knowledge a more scientific understanding of the genus may be possible. The "outer" part seems to be the canula or real end of the organ and the "inner" part only a lateral outgrowth from one side of the canula. The two are not of equal import.¹

¹ In practice stains that enter the canula tubule will aid in recognition of the canula without the need of sections.

The second stylets of *Astacus* and of *Cambarus* have the value of pleopods that have merely added a lateral outgrowth which arises in larval life and serves as an accessory organ in sperm transfer.

It is evident that the stylets of the species of *Cambarus* studied are much more highly specialized than the stylet of the American *Astacus* studied and this in turn seems less generalized than the *Astacus* of Europe.

The stylets of *Cambarus* could be readily derived from those of *Astacus* by specialization. The addition of glands, the strengthening of the shell, the refinement of the conducting tubule and the perfection of the accessory stylet in *Cambarus* may all be regarded as correlated with the presence of the annulus and sperm pocket in the females in this genus: the more accurate apparatus of the male *Cambarus* being used for a much more specialized task.

The first stylets in both genera might be derived from a flat stylet similar to that in the lobster where, probably, the two, right and left, are used at the same time to fill the sperm receptacle.

Upon this assumption we would regard *Astacus* as having lost some sort of a sperm receptacle which has been retained and perfected by *Cambarus*.

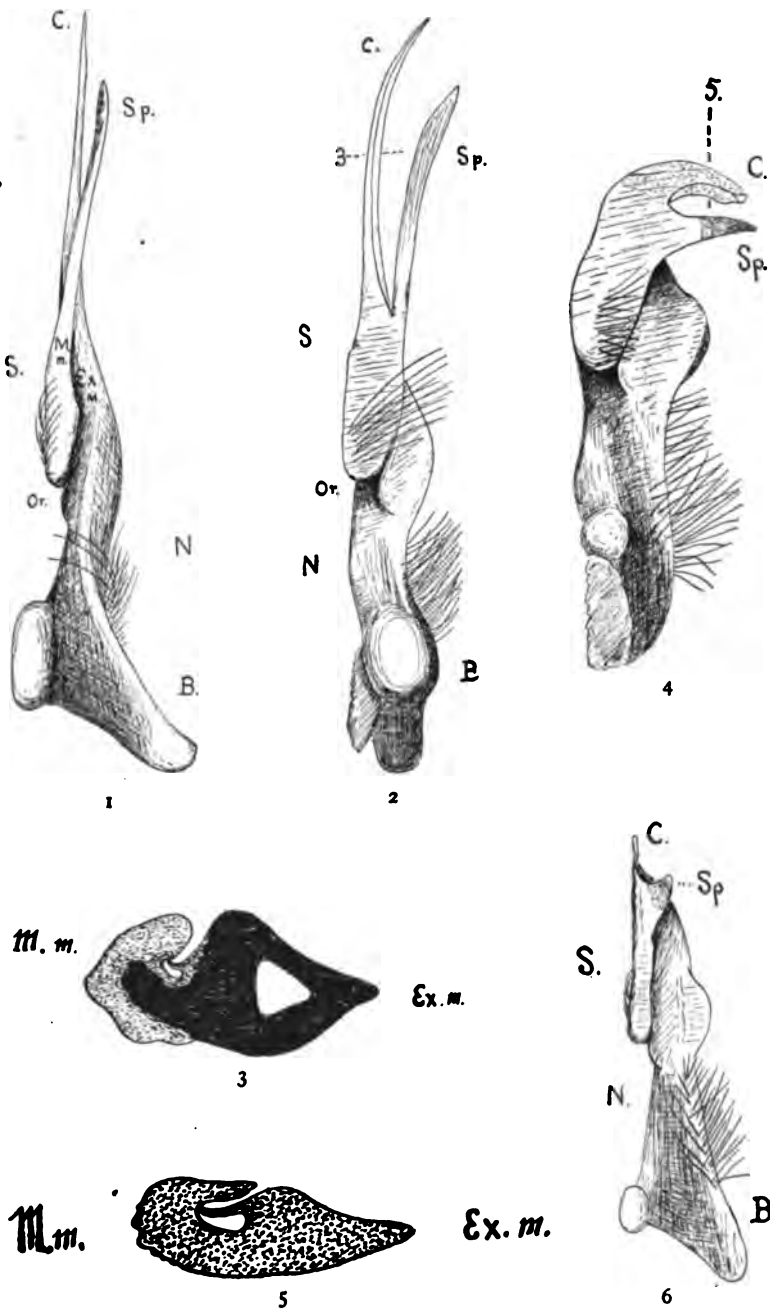
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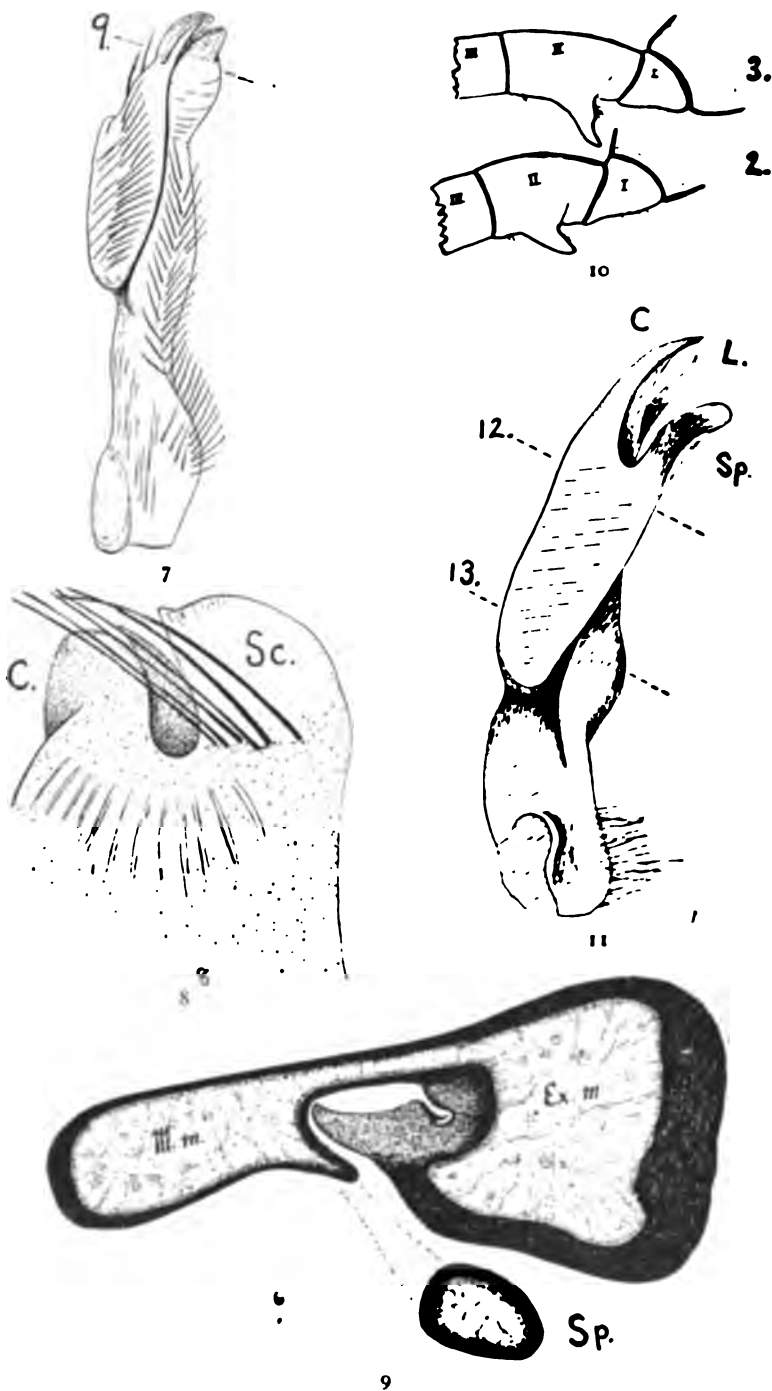
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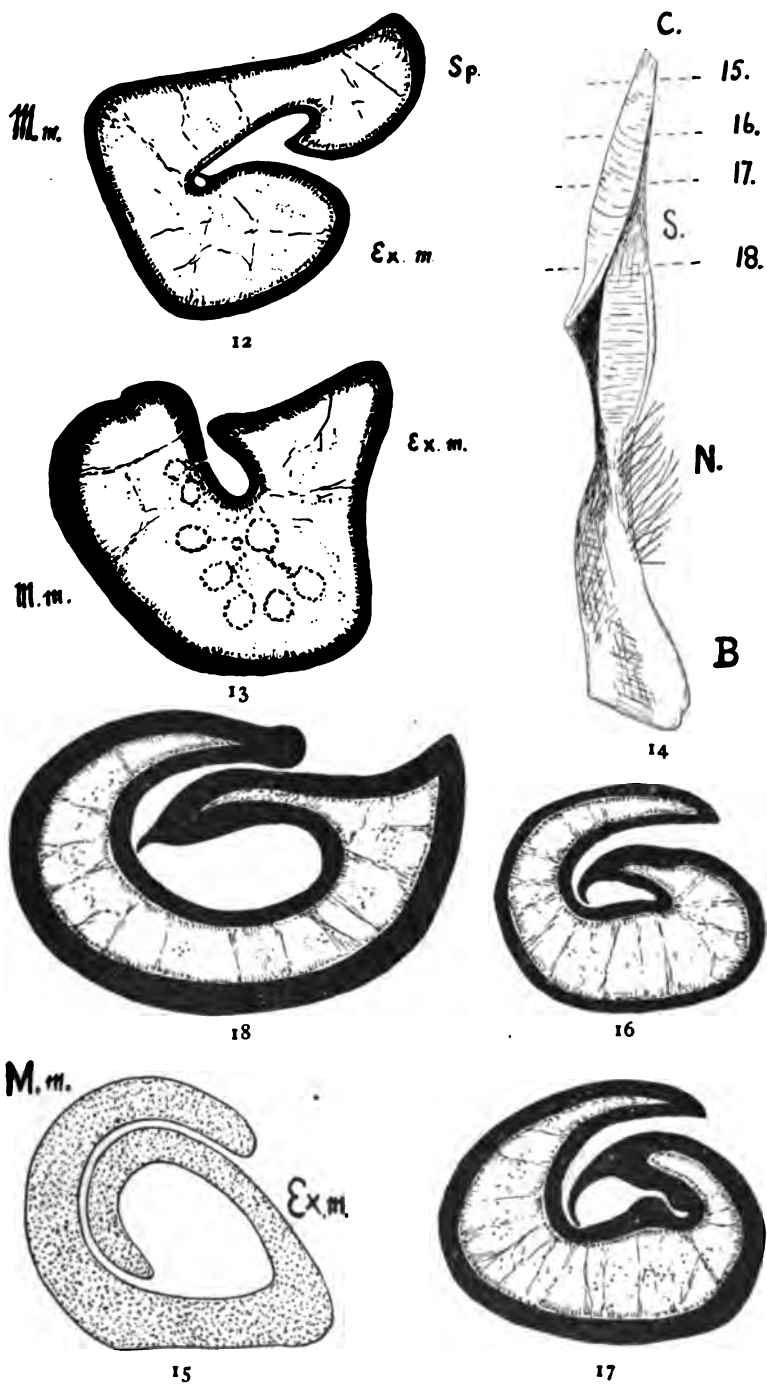
EXPLANATION OF FIGURES.

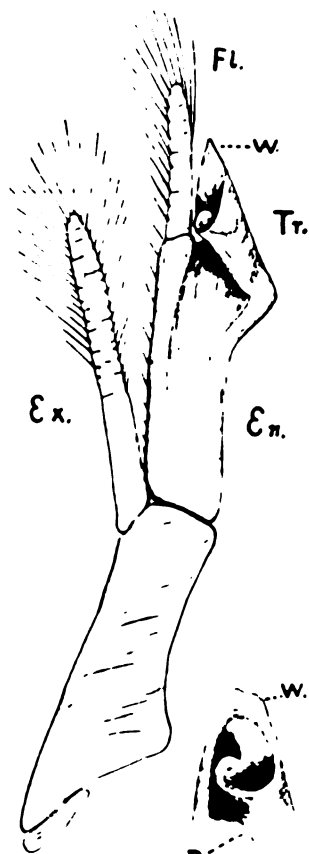
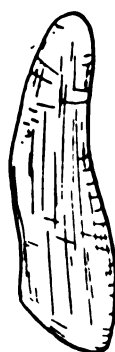
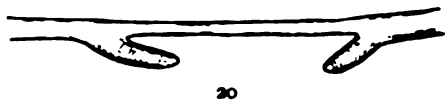
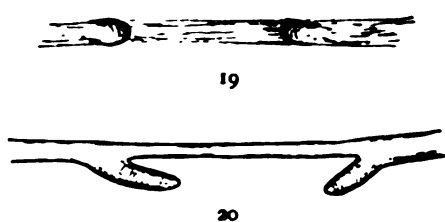
FIG. 1. *Cambarus virilis*: 1, posterior face of left stylet of adult, $2a_0$; 2, median face of the same, $2a_0$; 3, cross-section of the stylet at the level, $3pf$, Fig. 2, $2A$. *Cambarus Diogenes*: 4, median face of left stylet, $2a_0$; 5, section across the above at the level, 5 , $2A$; 6, posterior face of the left stylet, $2a_0$. *Cambarus Clarkii*: 7, median face of left stylet, $2a_0$; 8, enlarged view of external face of tip of left stylet, 2 , 90 mm., A ; 9, section across stylet at level, 9, of Fig. 7, $2A$. *Cambarus montezuma*: 10, bases of second and third left legs, showing hooks, of male 30 mm. long, anterior view, $2a_0$; 11, median face of left stylet, 2 , 90 mm., A ; 12, cross-section on the level 12 of Fig. 11, $4A$; 13, cross-section of same on level 13, $4A$.

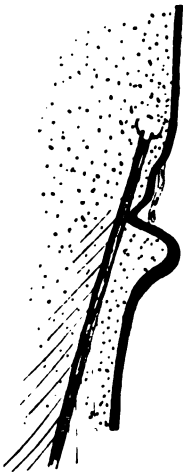
FIG. 2. *Astacus leniusculus*: 14, posterior face of left stylet turned to show somewhat of the median face, $2a_0$; 15, section across the above at the level 15 of Fig. 14, $2D$; 16, cross-section at the level 16 of Fig. 14, 2 , 90 mm., A ; 17, cross-section at the level 17 of Fig. 14, 2 , 90 mm., A ; 18, cross-section at the level 18 of Fig. 14, 2 , 90 mm., A ; 19, papillæ, or stylet on the first abdominal somite of larval shell passing from the fourth to the fifth stage. Length of body, 19.5 mm., 2 , aa ; 20, stylets of a male 20 mm. long, 2 , 90 mm., A ; 21, left stylet of a male 23 mm. long, $2A$; 22, stylet of a male like the last, $2A$; 23, left stylet of a male 25 mm. long, posterior face, $2A$; 24, second, or accessory, stylet; anterior face, somewhat turned to show the external face in part, $2a_0$; small figure to the right is the enlarged end of the radius; 25, part of the edge of the endopodite of the accessory stylet of a male 23 mm. long, anterior face, $2D$; 26, posterior face of the same, $2D$; 27, anterior face of the endopodite of the accessory stylet of a male 25 mm. long, $2A$; 28, cell outlines over elevation and pit of the edge of the endopodite of above stylet, $2D$.







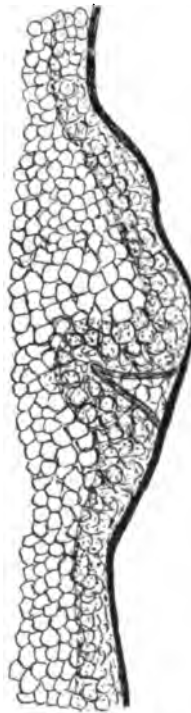




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BIOLOGICAL BULLETIN

A NEW GENUS OF PARASITIC GASTROPODS.

HAROLD HEATH.

A number of gastropods are known, living parasitically upon the body of certain echinoderms, which retain the shell and typical internal organization so that their systematic position is readily established. On the other hand several endoparasitic species exist which have become so highly modified that they stand in much the same relation to the free-living forms as *Sacculina* to the typical cirripedes. Owing to the lack of any detailed information relating to their ontogenetic development the relationship of such forms is highly problematical. And furthermore it is difficult to accurately follow the stages of the phylogenetic metamorphosis which the body has undergone, and accordingly to establish the homologies of some of the principal organs. Schiemenz¹ in a very suggestive paper has attempted to construct a hypothetical animal connecting the least modified species like *Stylifer* on one hand with the highly degenerate types represented by *Entoconcha*. In several respects the animal here described resembles the hypothetical form and in a measure enables us to follow some of the changes which the more degenerate species have undergone.

My attention was attracted to this gastropod by my friend and colleague Dr. W. K. Fisher who discovered it in a species of starfish (*Brisinga evermanni* Fisher) taken by the U. S. F. C. Str. "Albatross" in the neighborhood of the Hawaiian Islands (sta. 3467) at a depth of 310 fathoms. It occupied the coelomic cavity close to the base of one of the arms, producing a marked distention (Fig. 2, Pl. I.), of the body wall. The animal was un-

¹ P. Schiemenz, "Parasitische Schnecken-Kritische Referat," *Biol. Centralbl.*, Bd. 9.

attached and was put in communication with the exterior by a slit-like aperture 2 mm. long. The body is subglobular in form, distinctly bilateral, light yellow in color and measures 14 mm. by 11 mm. The external opening, communicating with the exterior through the aperture in the arm of the host, is in the mid line and is surrounded by prominent lips. These last named structures are covered with a firm cuticle produced into 16 pairs of interlocking teeth (Fig. 6, Pl. I.).

An examination of Fig. 1, Pl. I., will disclose the fact that the body proper, containing practically all the organs except the female reproductive gland, is overgrown by a great fold attached to the front end of the body, but elsewhere separated from it by a narrow slit-like space which communicates with the above mentioned fissure-like opening guarded by teeth. Anteriorly the ventral surface of the body is developed into a snout-like projection bearing the mouth opening and a small pair of tentacles (Fig. 1). More posteriorly the ventral surface, corresponding to the foot of free living forms, is somewhat flattened but lacks the usual high ciliated epithelium and gland cells. Still farther back the peculiar kidney forms the ventral surface behind which is the rectum borne on a papilla-like elevation. Some of these characters and others to be mentioned indicate distinctly gastropod relationships which have been retained in spite of parasitic habits.

The entire surface of the body is covered with a cuticular layer usually well developed on the ventral surface over the lobe-like projections shown in Fig. 8, Pl. I. In this last named region it becomes developed into numerous small papillæ each of which is penetrated by what is probably a nerve fiber (Fig. 7, Pl. I.). Within the animal the layer is much thinner and over the respiratory papillæ (ρ) and the adjacent regions is provided with numerous slender and apparently solid, hair-like processes.

The hypodermal layer consists of flattened cells, with large nuclei, separated at many points to allow muscle fibers to attach directly to the overlying cuticle. In addition there are many bipolar cells, one fiber passing distally into the above mentioned cuticular papillæ the other becoming lost in the subjacent tissue.

The mouth opening is situated upon the summit of a well-defined

proboscis which is concealed from view by a fold (not shown in figure) springing from its base. From the study of sections it is evident that this fold is of greater length than the proboscis and is not united along the median line posteriorly. Externally the fold is covered by a cuticular layer which is continuous over the proboscis and within the digestive tract itself as far as the radula. This last named organ is in a very rudimentary condition since it consists of but a single tooth (Fig. 5, Pl. I.) composed of some finely granular substance staining intensely in Delafield's hæmatoxylin. On each side of it are the openings of the salivary glands which are doubtless the homologue of the ventral pair in other molluscs.

The main portion of each salivary gland consists of a large sac located at the sides of the body proper as shown in Fig. 1, Pl. I. The component cells are highly vacuolated structures containing a faintly staining, granular secretion that is present also in the adjoining cavity. Ventrally, in close proximity to the outlet of this portion of the organ, the walls of the sac change abruptly in character and consist of more slender non-staining elements developed into low folds. Surrounding these cells are others of considerably greater height that form a papilla projecting into the cavity of the next division of the organ (Fig. 4, Pl. I.). This last named section is plain-walled and is composed of low columnar cells containing small quantities of a finely granular material, possibly a glandular substance differing from that of the larger sac. At the base of the proboscis the walls become produced into several small folds before entering the slender canal passing onward to its outlet at the side of the radula. Beyond the radula the pharyngeal or œsophageal epithelium becomes thicker and is attached to several muscle fibers acting as dilators and constrictors. The opening into the stomach is on the summit of a papilla whose general features are represented in text fig. A.

Opening into the œsophagus in close proximity to the stomach are two sets of glands, that may correspond to the dorsal salivary glands of other molluscs, whose position has shifted, though there is a possibility that they are strictly œsophageal products. Each group extends from the neighborhood of their outlet far

out into the pseudo-pallium, in some instances coming in contact with the hypodermal layer. So far as may be judged from the present specimen every cell possesses a slender ductule extending to its independent opening into the œsophagus. The secretion is colorless or of slightly pinkish tinge and in many cases is of less volume than the uniformly granular, strongly staining nuclei. In some instances the last named structures are more or less spherical, and again may be mammillated or formed of approximately eight globular masses as though formed by the incomplete fusion of as many chromosomes.

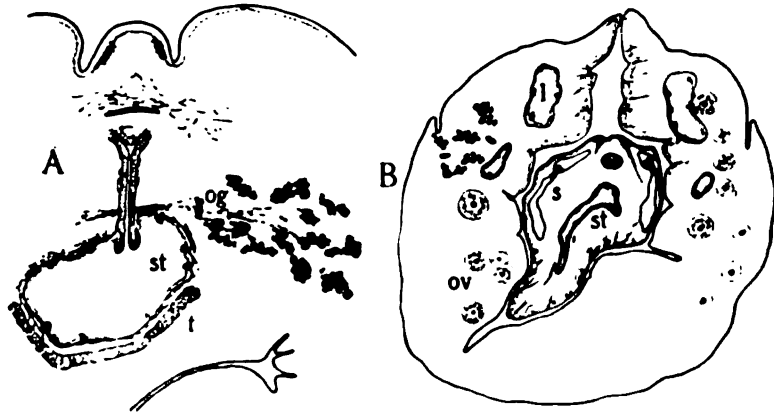


FIG. A. Section at level of œsophageal opening into stomach (st). og, œsophageal glands; t, testis.

FIG. B. Section corresponding to line A, Fig. 1, Pl. I. l, liver; ov, ova; s, salivary gland; st, stomach. The pleural pedal ganglion above (in figure) the stomach has, for the sake of clearness, been shifted slightly forward in Fig. 1, Pl. I.

The stomach is a voluminous sac extending throughout the greater part of the body proper. Its walls are composed of what appear to be two distinct types of cells (Fig. 3, Pl. I.) though they may possibly represent different stages of glandular activity. The more abundant form is almost cubical, highly vacuolated and contains a few slightly yellowish spherical granules. Among these are very much larger elements protruding some distance into the neighboring lumen and distended with a finely granular vacuolated material in which are a few spherical granules similar to those of the other type of cell. Opposite the level of the proboscis the stomach is expanded on each side to form a voluminous

pouch provided with a few secondary branches. These pouches probably are to be considered as representing the hepato-pancreas of other molluscs though the component cells differ in scarcely any essential respect from those of the main division of the stomach. At several points in the pseudo-pallium structures occur that strongly resemble the liver, but as they lack any definite connections it is impossible to claim that they were once united with the digestive tract.

The intestine presents the form of a cylindrical tube invested with numerous circular and longitudinal muscles attached by connective tissue and muscle bundles to the stomach and body wall. Its epithelial lining consists of relatively slender cells whose boundaries distally are difficult to determine owing to the large quantities of highly vacuolated protoplasm they contain. The outlet is guarded on each side by a large conical papilla covered with a thick cuticle fashioned near its tip into several slender filaments.

The stomach contains a small quantity only of a finely granular substance so that it is impossible to gain any insight into the methods of feeding and the nature of the food of this animal. It is probable that the proboscis and tentacles may be protruded through the external slit-like opening and organic substances may be picked up from the ooze as its host crawls about. It apparently absorbs little if any nourishment from its host though there may be some interchange of gases.

No sign of a heart exists in this species as is the case also with *Entoconcha*. A clotted substance abounds in the lacunæ among the various organs which doubtless represents blood. Groups of cells here and there may be corpuscles but their resemblance to connective tissue cells renders the determination uncertain.

As noted in a preceding paragraph respiration may be effected to a slight degree through the general body surface in contact with the coelomic fluid of the host. The chief respiratory organs however appear to be the finger-shaped processes (Fig. 1, *p*, Pl. I.) attached to the posterior end of the body. With the exception of the hypodermal layer and a few muscle fibers passing in various directions from wall to wall or attached throughout their entire extent to the walls these organs are hollow and are probably

more or less distended with blood in a living condition. It is probable also that they may be extended into the neighborhood of the external opening or even protruded through it.

A problematic organ, which appears to be a kidney, is situated beneath the stomach anterior to the intestine (Fig. 1, *k*, Pl. I.). In this region the body wall is provided with a considerable number of outpouchings of varying size, though usually comparatively short, each of which is invested with a definite cuticular layer. In life all of these are probably filled with blood and are lined with a few connective tissue and muscle fibers some of which may span the cavity. With the exception of a few of the anterior projections the walls are provided internally with a very considerable number of relatively slender finger-shaped processes resembling those on the inner wall of certain prosobranch kidneys. They differ, however, in being invaginations of the body wall and are lined with a continuation of the cuticular layer covering the body generally. The cells of these minor processes are of moderate size, more or less cubical, though they often become flattened near the tip of the organ, and under high magnification have been seen in several instances to connect with a central lumen by means of delicate pores. Judging from these appearances the cells of each of these slender processes extract from the surrounding plasma waste materials, and pass them into the contained lumen from whence they make their way to the exterior.

While the ganglia are readily located the nerves are not sharply differentiated from the muscle fibers through which they pass and accordingly have been traced in a few cases only. The cerebral ganglia, united by a relatively long commissure, are situated in front of the pharynx and beneath the forward end of the stomach (Fig. 1, Pl. I.). Two connectives lead backward at the sides of the pharynx to the pleuro-pedal ganglionic masses placed about opposite the level of the posterior border of the proboscis. These last named ganglia are indistinguishably fused though they are distinctly paired and originate a very few small nerves that have been followed for a short distance only. One large branch on each side, arising from the anterior half of the nerve mass, passes dorsally and laterally and breaks up into three divisions. The first passes up into the large fold or pseudo-pallium close to the

large cavity, ventral to the body proper, that communicates with the exterior. The second pursues a course anteriorly in the pseudo-pallium and disappears from view among the large ova. The third, also in the pseudo-pallium, makes its way backward for a considerable distance but finally vanishes in several small muscle bundles.

Together with *Entoconcha* and *Enteroxenos* this species is monœcious and the ovary and testis are separate and distinct. The testis occupies a position lateral and ventral to the stomach about opposite the level of the pharynx, and is bilaterally symmetrical and gives evidence of being a paired organ. In the region concealed by the liver in Fig. 1. Pl. I., the organ is continuous across the mid line; but anterior to this point the unpaired division develops an extensive anteriorly directed pouch on each side of the stomach. The gland is in an immature condition, containing many spermatogonia but no later stages. Behind the unpaired section a duct of very large caliber arises, on each side of the mid line, with thin walls and a glandular, apparently ciliated, low ridge on its inner face. In this condition it continues to a point opposite the middle of the kidney where it contracts abruptly to a slender, thicker walled tube which is directed ventrally to open into the narrow slit-like space between the body and some of the kidney lobes.

The ovary is confined to the large fold enveloping the body, practically all of the space unoccupied by the liver being filled with large ova. These are suspended, almost precisely as in the chitons (see Haller's Fig. 328, Lang's "Lehrbuch," p. 366), in slender sacs, outpouchings of the germinal epithelium, and in a completed condition are surrounded by a follicle. No definite oviducts are present, the ova probably escaping by means of ruptures in the wall adjacent to the body proper.

The ova, fertilized either in or out of the body, may readily escape from the animal and from the host and doubtless in the free-swimming stage make their way to another host into which they burrow and take up their final position. The changes that ensue are wholly unknown but it is evident that the fold or pseudo-pallium, in this species at least, is not a portion of the foot. As may be seen in Fig. 1 it arises as a duplicature of the

body wall in front of the proboscis and may in reality represent a modified mantle, having been developed from such a type as now exists in the aspidobranchs.

Concerning the systematic relationships of this animal it is difficult to speak with certainty. From the paired character of the testis it appears to have been derived from some aspidobranch ancestor in which there were traces of the original bilateral symmetry. In its present condition the animal is perfectly symmetrical, but it does not follow that its immediate ancestors were such. Just as the modern more or less symmetrical lithodes have been derived from the asymmetrical hermit crabs so this species may have secondarily assumed its symmetrical condition.

The genus may be defined as follows :

Ctenosculum new genus. Body globular, bilaterally symmetrical, almost completely enveloped in a fold of the body wall. Proboscis and tentacles present. Two sets of salivary glands, radula reduced to one triangular tooth. Monœcious, testis and ovary distinct and separate, the latter occupying the fold. Parasitic in starfish, *Brisinga evermanni*, Hawaiian Ids. Type of genus *C. hawaiiense*.

C. hawaiiense new species. With characters of the genus.

EXPLANATION OF PLATE I.

FIG. 1. *Ctenosculum hawaiiense* with left half of the pseudo-pallium removed. *c*, cerebral ganglia; *k*, kidney; *l*, liver; *o*, oesophagus; *p*, papillæ arising from body wall; *sg*, salivary gland; *st*, stomach.

FIG. 2. Cyst in starfish arm produced by parasite.

FIG. 3. Two types of cells of gastric epithelium.

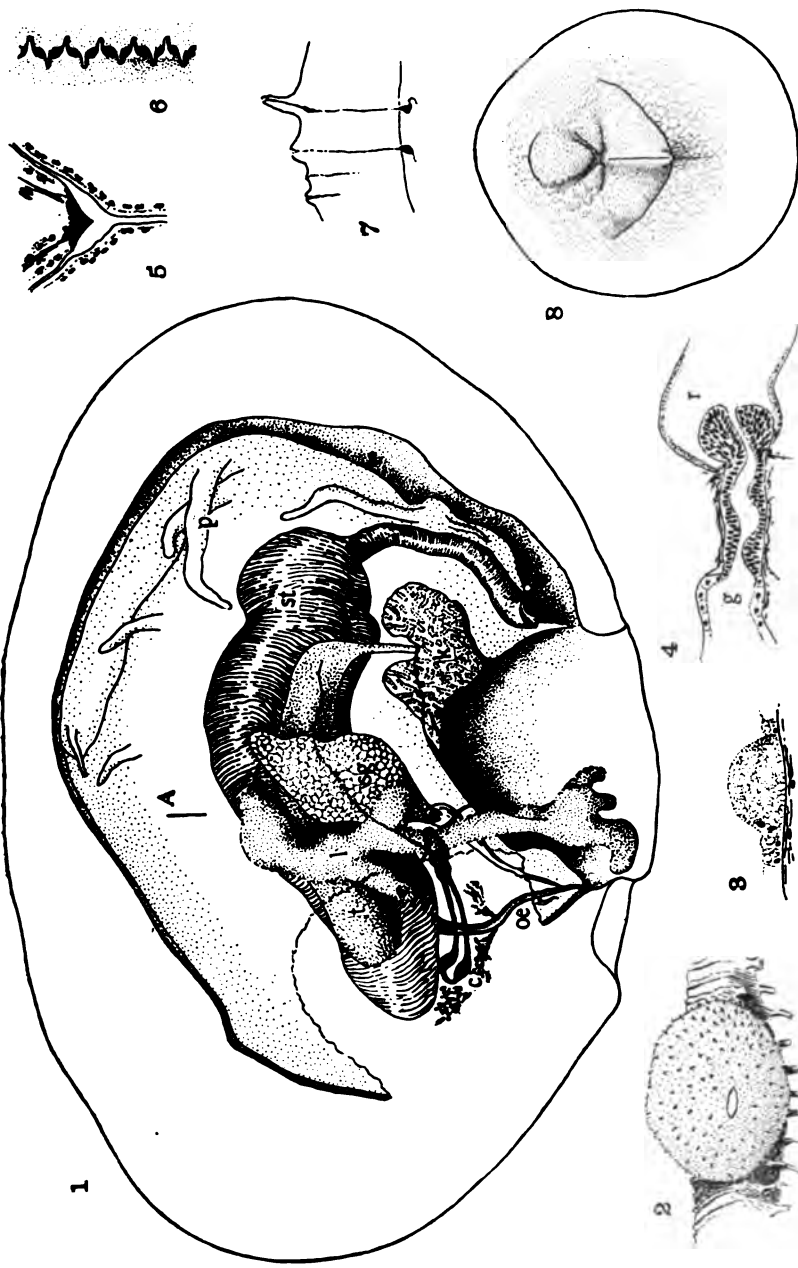
FIG. 4. Outlet of salivary gland (*g*) into reservoir (*r*).

FIG. 5. The single tooth of the radula with salivary ducts opening on each side.

FIG. 6. Cuticular teeth guarding the opening into space between body and pseudo-pallium.

FIG. 7. Papillate cuticle, from ventral surface of body, traversed by nerve fibers.

FIG. 8. *C. hawaiiense*, entire animal, ventral view.



THE OCCURRENCE OF AMITOSIS IN MONIEZIA.

C. M. CHILD.

In a recent paper, "On the Method of Cell Division in *Tania*," by A. Richards,¹ the author records his failure to find amitotic division in *Tania* and suggests on the basis of his results that my conclusions concerning amitosis in *Moniezia*² are due to errors of observation.

The appearance of this paper makes it necessary for me, in defence of my observations, to emphasize certain features of my own work, to which Richards has apparently not given adequate consideration, to add a few observations not included or only briefly referred to in my earlier papers and finally to subject his attempts at interpretation to critical analysis.

In the first place, it might seem that the reading of my statement concerning methods of fixation and precautions employed (I., pp. 89-93) would suggest to anyone that I had exercised at least some degree of care in order to avoid errors of observation of so gross a nature as the mistaking of a mass of "Nebendotter" for a nucleus. As I stated, fresh material was fixed by the most various methods during each of four successive years, and a large part of my time during this period was devoted to the study of the preparations, from which hundreds of camera drawings were made. The work was begun simply because the apparent absence of mitosis in certain regions of growth interested me, and I expected to find either some unusual type of mitosis or else short recurrent periods of mitosis. What I did find, however, led me to exercise the utmost care and to go over the ground repeatedly even after I had published my preliminary paper in 1904.³ During the whole period of my work I was constantly searching for

¹ BIOL. BULL., XVII., 4, 1909.

² Child, "Studies on the Relation between Amitosis and Mitosis," I., BIOL. BULL., XII., 2, 1907; II., *ibid.*, 3-4; III., *ibid.*, XIII., 3, 1907; IV. and V., *ibid.*, 4. In later references to these papers in the text they will be designated simply by the Roman numerals I.-V.

³ "Amitosis in *Moniezia*," *Anat. Anz.*, XXV., 22, 1904.

modified forms of mitosis as a possible basis for interpretation, but the frequency and almost diagrammatic clearness of the cases which I could interpret only as direct division, satisfied me that no such interpretation was possible. I have pointed out (I., pp. 91-2) the difficulties attendant upon the observation of amitosis in fixed material and the methods employed for giving the greatest possible certainty. Cases of apparent division which appeared in the slightest degree doubtful were not accepted as evidence. I have demonstrated many cases of apparent amitosis to others, both students and colleagues, who were able to see them with perfect clearness and had no doubt of their being what they seemed to be.

On pp. 312-313 of his paper Richards says: "I must protest against the balancing of results obtained from such unfavorable material as that which cestodes offer against such favorable objects for cytological study as for example the Orthoptera." This protest involves somewhat peculiar ideas of cytological study. Apparently, according to Richards, we must draw our conclusions from "favorable" material and leave the unfavorable aside. And apparently also because certain phenomena are clearly visible in favorable material, *e. g.*, the Orthoptera, we must conclude that the phenomena in other less favorable material are essentially similar. No field of biological science, and perhaps no field of science in general, has suffered to such an extent as has morphology from premature generalizations based on observation of one or a few forms. Indeed it is perhaps not too much to say that limited observation and premature attempts at generalization often go hand in hand.

As regards the "unfavorable" character of the *Moniezia* tissues I must disagree to some extent with Richards. With proper fixation, with sections sufficiently thin — most of mine were 2-3 micra — and with careful staining and extraction, the material is no more difficult to study, at least as regards nuclear phenomena, than many other tissues. I have seen hundreds of cases of apparent amitosis which were almost diagrammatic in their clearness when observed with a little care, though of course not as conspicuous as certain phenomena of mitosis in certain species and cells. If observation of fixed and stained material is

of any value at all as a means of discovering the visible phenomena of cell division, I can only conclude that direct division occurs in *Moniezia* as I have stated and that it can be seen readily enough by anyone who is willing to take sufficient care in preparation and observation.

Moreover, I have not, so far as I am aware, attempted at any time to "balance" my observations against those of others. The real point at issue is not my observations against those of others, but my observations against a hypothesis, which cannot be proved by observation, viz., the chromosome hypothesis, for the only real objection to the acceptance of my observations at their face value is the existence of this hypothesis. I do not wish to enter into fruitless discussion of the hypothesis, but merely to emphasize the fact that I have attempted in every way possible to make the record of my observations a record of actual fact. Such records cannot be controverted either by observations on another genus and species or by invoking a hypothesis which is far from being proved. It is interesting to note that Richards' paper is to a considerable extent devoted to "balancing" his observations against my own, although both according to him are made on the "unfavorable" cestode material.

Turning now to certain matters of detail, we may consider first certain points with respect to the parenchyma. My observations on amitosis began with a study of the neck-region, *i. e.*, the region between the scolex and the first visible proglottids. In this region the tissue of the body consists mainly of parenchymal cells, muscle fibers, the nerve cords and the nephridial canals. At the posterior end of this region the proglottids become visibly marked off one after the other, each including a certain portion of the tissue. In other words, the cells which constitute each proglottid either preëxist, or arise by the division of preëxisting cells in the neck-region, or else they must arise *de novo*. Examination of transverse sections at short intervals from the scolex backward into the proglottid region shows a very considerable increase in the number of nuclei, as I have determined by actual counts of the nuclei in the sections. In short there can be no doubt that new parenchymal nuclei are formed in this region, and when we consider the enormous number of proglottids formed

from a single neck-region and observe that the number of nuclei in the neck-region at any given time is sufficient for only a few proglottids it becomes evident that during the life of the animal an enormous number of new nuclei is formed in some way in this region. These facts dispose of Richards' conclusion that the parenchyma grows chiefly by the formation of "intercellular material."

Moreover, in view of these facts it is not in the least surprising that Richards has failed to observe any division, either mitotic or amitotic in the parenchymal cells (p. 323), since, so far as can be determined from his statements, he has apparently paid little or no attention to the neck-region. Mere observation of the method of growth and the abundance of nuclei in this region forces us to conclude, either that nuclear divisions occur in enormous numbers in this region or else that nuclei are formed *de novo*, an alternative which most of us would hesitate to accept without proof of the most conclusive character.

When one examines scores of these neck-regions, as I have done, and finds first absolutely no mitoses and second hundreds of cases of apparent amitosis, many of them almost diagrammatic, the evidence for the occurrence of amitosis acquires at least some value.

On p. 322 Richards says: "Child has assumed all through his work that the absence of mitotic figures in tissues known to be growing rapidly is evidence of the occurrence of division by amitosis." He then proceeds to show that the growth of the parenchyma occurs to a large extent by the formation of "intercellular material." This statement of Richards seems, so far as it concerns my own position, to be an error. My argument is actually as follows: When mitosis is absent from regions where rapid increase in the number of nuclei is manifestly taking place, we have good reason to believe that some other form of nuclear division is occurring, especially when we have discovered what appears to be this other form in other tissues of the same animal.

I have, I think, made it sufficiently clear all through my work that in speaking of regions of rapid growth I meant not merely regions in which the cells were increasing in size or forming intercellular material, but those in which they were actually increas-

ing in number. I have never, however, considered absence of mitosis in such cases as proof of the occurrence of amitosis. Only the actual observation of amitosis can prove that it is occurring.

I wish to add to my earlier observations the fact that in a single individual consisting of scolex, neck-region and a few of the youngest proglottids, I have found almost every nucleus in mitotic division. This is the only case of the sort which I have ever observed. Aside from this I have never seen a single case of mitosis in this region of the body. I am inclined to believe that this is a young animal in the early stages of growth. In my work on various other forms, as yet unpublished, I have found considerable evidence in support of the view that amitosis becomes more frequent as growth (with cell division) becomes more rapid. In *Planaria*, for example, regeneration begins with mitosis, but as the growth of the new tissue becomes more rapid amitosis takes the place of mitosis. Patterson¹ has found that the frequency of apparent amitosis increases in regions of rapid growth, and other evidence to be presented elsewhere exists.

For those to whom the positive observations on amitosis are of little or no value as evidence this case of mitosis in the neck-region of *Moniezia* opens a way for simple interpretation. They will conclude, as Richards suggests (p. 320), that mitosis is of short duration and occurs in waves, *i. e.*, is periodic, and that all the other individuals observed by me are simply stages between two such periods. At present, however, I cannot accept such an interpretation as the correct one. Not only the cells of the neck-region, but the cells of the scolex, which does not take part in the growth of other regions are undergoing mitosis in this specimen. Moreover, I am as yet unable to convince myself that the many cases of apparent amitosis, which I have observed, in the neck-region of other individuals, are all errors of observation, or something else than nuclear division.

Concerning the structure of the nuclei in *Moniezia*, it may be said merely that the non-chromatic portions of the "embryonic nuclei" present widely different appearances with different fixing agents. After Hermann's fluid the nuclear structure of the primitive germ nuclei and the parenchyma nuclei is somewhat similar

¹ "Amitosis in the Pigeon's Egg," *Anat. Ann.*, XXXII., 5, 1908.

to that figured by Richards for *Tania*, but after comparing the results of various fixing agents I came to regard it as highly probable that the "strands of linin" were simply coagulation products and therefore stated (I., p. 93) that "a distinct reticular structure does not appear." Richards (p. 316) attributes to me the statement that "the nuclei do not contain any definite reticulum." Though I am not desirous of splitting hairs this seems to me a much more positive statement than that which I did make. I do not know how widely different the nuclei of *Moniezia* are from those of *Tania*, and while I could give figures showing "strands of linin" in some of these nuclei, I am strongly skeptical, for the reasons given above, as to their representing in *Moniezia* a real nuclear structure existing before fixation. As the germ cells approach maturation the visible nuclear structure undergoes alteration, as my figures show, and the non-chromatic portions show more definite and constant characteristics. Richards in his work has apparently employed only Flemming's and Zenker's fluids, both of which will, under certain conditions, produce reticular structure in solutions of proteids. Since the "strands of linin" were sometimes confusing in the study of the nuclear membrane I often found it desirable to extract the stain to such an extent that they were only slightly stained, or to employ fixing agents which did not show them in the resting nuclei.

But the chief interest centers of course about the occurrence or non-occurrence of amitosis in the germ cycle. It is evident that if all the nuclei which are included in a given proglottid arise, or may under certain conditions arise, in the neck-region by amitotic division the germ cells must arise from nuclei which have previously divided amitotically. This conclusion is, however, open to the objection that since we cannot be certain that every individual nucleus in the neck-region has divided amitotically, it may be possible that the nuclei of the germ cycle have not so divided. This objection is actually of little force, for there are probably not enough parenchymal nuclei in the neck-region at any one time to give rise even to all the mother germ cells which appear. Nevertheless this objection must be reckoned with and I should not for a moment maintain that the amitotic division in the neck-region constitutes absolute proof of the occurrence of amitosis in the germ cycle.

In my own work I proceeded posteriorly from the neck through the youngest proglottids to the region in which the reproductive organs first appear, and then followed their development from this point to and through the process of maturation. In the primitive germ cells mitosis is more or less frequent, as I have shown in my earlier papers, and if I had not found the most positive evidence of the occurrence of amitosis I should not have doubted that in this period cell division was wholly mitotic. But I found it is impossible to convince myself that this was the case, simply because I observed too frequently what I could not interpret as anything but amitosis.

Richards has not found amitosis in the germ cells, but he does not state what stages in the development of the reproductive organs he has examined. If one may judge from his figures and his statement on p. 313 that "the female sex cells in the cestodes in question are by far the largest cells in the body," it would appear that he has examined only the later stages, in which the cells have already assumed the definite characteristics of germ cells. In these stages I myself have never been able to observe amitosis with certainty, though mitosis is often seen.

It is during what one might designate as the embryonic development of the reproductive organs, when the germ cells, at least in the female, are not visibly different, from the cells which form the vitellaria and the reproductive ducts, that I have found amitosis, though even in these stages mitosis sometimes occurs. In order to forestall a possible objection, I should perhaps call attention again to the fact already noted in my earlier work (I., pp. 97-109) that after certain stages the portions which are to form the ovary can readily be distinguished from those which are to form vitellaria and ducts by their position and method of growth, though the cells are all still apparently similar.

Richards says absolutely nothing concerning these earlier stages in his work on *Tænia*, and until further evidence is forthcoming, it seems at least possible that his failure to discover amitosis may have been due to the fact that he examined only stages where it does not occur. However, I know nothing from personal observation as to the occurrence or non-occurrence of amitosis in the species which Richards has examined, and since

I am inclined to believe that the relative frequency of mitosis and amitosis in certain species, and even in single individuals, *e. g.*, *Planaria* may vary greatly according to conditions, it is also possible that Richards' material differs more or less widely from mine.

And now a few words concerning Richards' attempts at interpretation of my observations on the germ cells. On p. 315 he suggests that I have been misled by mistaking for a nucleus or part of a nucleus a mass of "Nebendotter" which occurs in "some of the early oögonia . . . while it is not present in some oöcytes" (p. 313). That one investigator working with one species should be willing, without more positive evidence than Richards has anywhere brought forward, to attribute to another, of wider experience than himself, and working with a different species and genus, so gross an error of observation as this is, to say the least, somewhat surprising. But the facts are these: as I have noted above, amitosis occurs in the earlier stages of development of the ovary and in such stages the primitive germ cells certainly contain no "Nebendotter." As a matter of fact, I have never seen anything of the sort at any stage in the ova of *Moniezia*, although the cells of the vitellaria, which in certain stages may easily be mistaken for ova, develop such masses rather early in their history, but usually not until division has ceased (see Child, I., Figs. 31-35, and pp. 112-3). I cannot see that my accounts of nuclear structure and division afford any possible basis for the relief that I could have mistaken for a nucleus a body which according to Richards stains readily with iron hæmatoxylin and appears "as a dark homogeneous mass even after a great deal of extraction," by which "nucleus and cytoplasm may be entirely decolorized" (Richards, p. 314). Moreover I have employed various methods of staining which leave the yolk almost wholly unstained.

Concerning the slight difference in color between the two parts of a nucleus, which I noted occasionally (Child, I., p. 95), it need only be said that I have often observed such differences in the somatic cells of *Moniezia*, as well as in the somatic cells of other forms, where there can be no question concerning "Nebendotter," consequently Richards' suggestion (p. 315) that

one of the parts in such cases is "Nebendotter" falls to the ground.

As regards Richards' interpretation of "endogenous" division (Child, I., p. 95), which he presents in connection with the case of mitosis shown in his Figs. 17 and 18, it may be said that such attempts at interpretation of another's observations are scarcely worthy of criticism. The statement of an author that he has employed so far as possible every precaution and has repeated his observations again and again to avoid error, is ordinarily given some degree of consideration. Moreover, as regards this particular point I stated (Child, I., pp. 95-6) that "some cases of this sort of almost diagrammatic clearness have been observed." And finally reference to a few figures (*e. g.*, I., Fig. 13E; II., Figs. 9A and 9C) is sufficient to show that the parts in such divisions are often very different in size and the membranes perfectly distinct throughout. There is then not the slightest basis for regarding them as misinterpreted mitoses.

To sum up: Richards' evidence for the non-occurrence of amitosis is wholly negative, my evidence for its occurrence is positive. How far his failure to find amitosis may be due to failure to examine the proper stages and how far to the actual absence of such phenomena as I have described, it is impossible to determine. Second, his attempts at interpretation of my results as errors of observation are wholly unsuccessful, since they concern stages of development very different from those on which I have based my conclusions and occurring in another genus, and since they involve a total disregard of much that I have said concerning methods, precautions, the appearance of the nuclei, etc.

The whole question of the occurrence or non-occurrence of amitosis is one which requires great care and extended research, and it may be doubted whether it is a suitable subject for a beginner in cytological investigation, both because of his lack of experience and because he is likely to be guided to a greater or less extent by the views of his instructor.

I wish to add a brief remark concerning my later observations on amitosis in other forms.¹ In this paper I merely recorded the

¹ Child, "Amitosis as a Factor in Normal and Regulatory Growth," *Anat. Anz.*, XXX., 11 and 12, 1907.

occurrence of what seemed to me to be undoubted cases of amitosis in a number of species from different groups of the animal kingdom, without attempting in most cases to determine its frequency or significance, and added a brief consideration of the physiological and theoretical significance of amitosis in general. Concerning my observations in this paper Richards says (p. 311) that my "evidence is lacking in some respects." I scarcely see how other evidence than positive statements and figures can be given and must suggest the desirability of more definite criticism than this, for in discrediting another's observations it is no more than just to present definite reasons for one's position.

And finally: if my observations are not in accord with certain current hypotheses it is not because of any preconceived opinions on my part concerning those hypotheses, but simply because the results of my work have forced me to believe that the hypotheses are not in accord with all the facts. As regards this point, it would seem that our present knowledge of regulatory phenomena in organisms is sufficient to show very clearly that certain results may be attained in different ways under different conditions. Moreover, the periodical recurrence of specific structures in definite form and number is one of the most characteristic features of the organic cycle, but we do not regard it as necessary to believe that those structures should persist as distinct and continuous entities during the periods when they are not visible. To speak specifically, why should we regard the chromosome problem as fundamentally different from the problem involved in the recurrence in each generation of five fingers upon a hand, or the regulatory development of a definite and characteristic number of tentacles in a piece of an actinian body? The farther we proceed in our physiological analysis of developmental phenomena, the more evident does it become that the finger and the tentacle do not persist as distinct and definite entities during all the period when they are not present as visible structures. The only thing which persists is the physiological capacity to react in a certain way under certain conditions, and when these conditions arise the characteristic structure-complex appears.

In short, when we consider the chromosome problem from a physiological instead of a purely morphological point of view, and

we must consider it in this way sooner or later, we can find no good reason for regarding it as fundamentally different from many other problems of ontogeny. Physiologically it is no more difficult to conceive that a piece of a nucleus should, under certain conditions, give rise to the characteristic number of chromosomes, than that a piece of the actinian body should give rise to the characteristic number of tentacles. The correctness of the chromosome hypothesis is far from being demonstrated, and in view of this fact we must consider the possibility of revising the hypothesis to fit the facts, as well as that of bringing the facts into accord with the hypothesis.

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THE MANIFESTATION OF THE FLIGHT-FUNCTION IN THE SILKWORM (*BOMBYX MORI*).

ISABEL McCracken.

The activities of the adult silkworm moth (*Bombyx mori*) are normally confined to those connected with the reproductive reflexes, mating and ovipositing.¹ The moth issues from its cocoon sexually mature and lives in a perfectly functional condition for several days without taking food and scarcely wandering from its birthplace. Its thoroughly domesticated condition and associated habits call for the exercise of but few of the usual insect instincts. In particular, searching for food and for mates having been dispensed with, the flight-function is a useless function and rarely manifest.

In general appearance, the newly emerged moth is a perfectly formed insect. A close inspection, however, shows vestigial mouth-parts, the taking of food having thereby become an impossibility. Otherwise, the various anatomical features are normal. The wings, particularly, are of perfect form and under perfect control so that the exercise of the flight-function is a possibility, though as stated the insects are rarely observed to fly.

"The presence of an organ," says Folsom,² "normally implies ability to use it. The newly born butterfly needs no practice preliminary to flight." The silkworm appears, from general observations, to be an exception to this rule. At any rate the non-flight habit has seemingly become fixed, absolutely so in the case of the female, but in the case of the male with exceptions and under special conditions to be herein noted.

This non-flight habit is such a common observation among silkworm breeders that investigators have made use of the wing of the silkworm moth to study indications of its structural degen-

¹ Kellogg, "Some Silkworm Moth Reflexes," *Biol. Bull.*, V., pp. 152-154.
McCracken, "The Egg-laying Apparatus in the Silkworm as a Reflex Apparatus," *Jour. of Comp. Neur. and Psychol.*, XVII., pp. 262-285, 1907.

² Folsom, "Entomology," p. 357.

eration on the basis of its being "functionally degenerate,"¹ and psychologists allude to the non-flight habit as indicating a loss of the "flying instinct."²

Especial interest therefore attaches to the fact that this apparently useless organ persists in a perfect form and under perfect muscular control.

Though slightly variable in the character of its venation, as pointed out by Kellogg and Bell, we do not know that the veins vary more than in the wings of moths of other species, and in comparing the wings of a long series of "non-fliers" with those of "fliers" I find no structural differences between the two lots. The wings appear, however, to be fundamentally non-useful, since both effectual mating and effectual ovipositing, the only adult activities, can be successfully accomplished without them. The de-winged silk-worm moth exhibits no inconvenience in these respects. Mayer-Soule³ found that in certain species males deprived of wings met with greater resistance from females when the females were deprived of sight. The female silkworm moth, however, exercises no selection between the normal and de-winged male, nor does the male between normal and de-winged female.

Although the wings are normally not used in flight, as pointed out, they are habitually fluttered preliminary to mating. This fluttering of the wings may effect a circulation of the air in the neighborhood of the moths so that the presence of the female with extended scent glands is more readily discerned by the male. However, the natural proximity of males and females under the usual conditions of silkworm breeding renders this use of but little importance.

The wings are also made use of in the "turning reflex." A moth placed upon its back instantaneously rights itself by bringing the forewings quickly together and thus exerting a pressure upon the surface. This procedure follows in the decapitated as well as in the normal moth, and though one rarely necessary under usual conditions it shows the perfect muscular and nervous control under which the wings are held.

¹ Kellogg and Bell, "Inheritance in Silkworms," I., Stanford Univ. Pub., No. 1, 1908.

² Oppenheim, "Mental Growth and Control," 1902, p. 99.

³ Mayer-Soule, "Some Reactions of Caterpillars and Moths," *Jour. Exper. Zööl.*, Vol. III., 1900.

Since, therefore, the wings are present and are under perfect muscular and nervous control, what is apparently missing in the normal moth is the flight "instinct," or the disposition to set in motion those reflexes that make for flight.

A study of the habits of the male silkworm moth has been made, therefore, with special reference to the flight function and some interesting suggestive conditions have been observed.

As previously stated, the mating instinct is strong in the young male. It is at once aroused if a female is near and the numerous reflexes that effect mating are initiated. Upon leaving its cocoon, under ordinary conditions, the male seeks the near-by female. After several hours in copula separation takes place. The female then begins to oviposit, and the male seeks another female and thus passes from female to female throughout the few days of its existence. Thus we may say that the mating instinct once aroused is the dominating and controlling instinct. In the absence of the female the male behaves somewhat differently. If a young male moth is isolated in a closed box and in a room with no females present, it remains for several days in a perfectly passive condition, scarcely moving. On the fourth or fifth day the moth begins to flutter wildly about within the box, and upon removal of the cover soars into the air, circling about and sustaining itself upon the wing for from thirty seconds to several minutes. It comes to rest seemingly from fatigue and may, after a few seconds, during which the wings are vigorously fluttering, again take flight for a few minutes finally coming to rest again to remain wherever chance may find it until death ensues.

Having by chance observed this behavior in several male moths, I selected, at the height of the breeding season, a large number of males as they issued from their cocoons and placed them in a large well-lighted room by themselves, some individually within closed boxes and others exposed each by itself on a small tray. In many cases the moth remained perfectly quiet for several days, then wandered about with a fluttering of the wings, finally coming to rest and remaining in a quiet condition until death. By far the greater number, about seventy-five out of one hundred, behaved as in the cases previously noted. They fluttered about vigorously on the fourth to the eighth days and then soared upward.

Thus it was ascertained that many male moths were not powerless with respect to flight. The moth has not "forgotten" how to fly. It would appear, however, that, during the first few days of the life of the moth the flight reflexes are under inhibition and remain so during the exercise of the mating reflexes. Restrained from mating, however, as has been demonstrated, the flight instinct asserts itself, and the flight reflexes are "let loose" as it were. Neither natural selection, which by carrying a "flier" far from a mate, and thus leaving it without offspring, nor the discontinuance of the need for flight have served to eradicate the possibility of exercising the flight function.

As stated, the moths thus far experimented with were deprived of mates. Later, a large series of moths was isolated after mating had taken place. They were first allowed to mate normally. When they had voluntarily left the female they were isolated as before. The result was the same. When first isolated they were quiet. After several days had elapsed a large percentage entered into the "fluttering" condition and flight ensued.

During the very beginning of the "fluttering" condition if a female is brought near, the male is attracted to her and mating takes place. If, however, the fluttering condition is well under way the male pays no attention to the female but flutters past her or over her, it may be, and finally takes to wing.

It would seem, therefore, from the foregoing observations that the flight reflex is, at the time the insect leaves its cocoon, part of the moth's sum-total of inheritance but is merely under inhibition.

The mating instinct is, however, exercisable at once, that is, is immediately dominant. A sufficiently long inhibition of the mating instinct appears, in a large number of individuals, to augment the flight reflex. Furthermore, with the flight reflex released, the mating reflexes are, for the time being, inhibited, or at least manifestation of the flight reflex takes place only during suspension of the activities of the mating instinct, and during the manifestation of the mating activity, cannot be aroused.

This manifestation is wholly, however, without purpose, is purely non-adapted. It is a manifestation apparently of what

Wheeler refers to as "latent heredity."¹ "The vestigial instinctive action," says Wheeler, "presents itself as an act of racial or phyletic recollection and must, like the representations of individual memory, depend on psychological dispositions abiding in latency, just as the visibly morphological characters of the adult organism arise from the visibly physiological dispositions in the germ plasm. These dispositions must be inherited with great tenacity and persistency, since vestiges, both instinctive and structural, often remain latent for generations and then suddenly manifest themselves under stress of extraordinary stimuli."

In the case at hand, the "extraordinary stimuli," whatever these may be, that produce the manifestation are not external. The movement has every appearance of being "spontaneous," the release of "internal energy." The behavior here noted is strikingly parallel to that noted by Jennings with reference to lower organisms. "Often, perhaps usually in lower organisms," observes Jennings, "movement in a certain direction is due only to the release of inhibition. The organism moves in a given direction because it is moving from internal impulse, and because movement in this direction is not prevented."² Jennings observes further, that "the behavior of the lower organism at any moment depends upon its physiological state at that moment."

Flight in the silkworm, manifesting itself as it does, without external stimulus and when the activities of the mating reflexes are suspended, is apparently a spontaneous movement dependent conceivably upon the physiological condition of the insect for the time being, and involves the awakening of a latent instinct.

In the presence of the female, the whole attention of the insect is monopolized for the time being with the impetus to mate. This "state" supercedes every other possible state and prompts or guides the insect to one line of conduct only. The action which is the normal consequent of this state is mating; all other possible actions are suppressed, and under normal conditions, as stated, habitually suppressed. The manifestation of the flight function under these experimental conditions shows that, though

¹ Wheeler, "Vestigial Instincts in Insects and other Animals," *Amer. Jour. of Phys. Zool.*, Vol. XIX., pp. 1-13.

² Jennings, "Behavior of Lower Organisms," p. 284.

this function is a part of the sum total of inheritance, the overwhelming development of the one instinct, that of mating, serves habitually to inhibit its activity. On the other hand, something has preserved both the wing structure and the flight function in spite of persistent non-use and natural selection, and although these have no relation to the preservation of the race in its present-day activities.

In the general conception of "instinct," its failure means extinction of the species.¹ In the case at hand, on the contrary its manifestation would mean extinction of the species. Its failure of manifestation is associated with the present-day life of the insect. Its manifestation under conditions herein observed attests the persistence in heredity of an ancestral function long after it has lost everything of a purposeful nature.

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¹ Jordan and Kellogg, "Evolution and Animal Life," p. 430.

A MATHEMATICAL TREATMENT OF SOME BIOLOGICAL PROBLEMS.

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Dr. King's work ('07, '09), "On Studies on Sex-determination in Amphibians," suggests an interesting problem which may be put in the following form :

A jar contains a large number of male and female tadpoles, the proportion of each being unknown : if on picking out $m + n$ tadpoles, m are found to be males and n females, to find the probability that the ratio of the number of either sex to the entire lot lies between given limits.

It will be seen that problems of this nature occur frequently in biological investigations and that it is of importance to have some method for determining the accuracy of the observed proportions. This can be done by means of the formula given below. As the development of the formula is somewhat complicated I shall present the entire process of the mathematical treatment of the solutions based on the theorem of Bayes, together with one application. Although such an elementary exposition of the subject will be superfluous for one who is familiar with the theory of probabilities nevertheless for others it may be helpful.

If p denote the probability that an event will happen, then $(1 - p)$ is the probability that the event will fail. If the probability that the event will fail on any single trial is $(1 - p)$, the probability that it will fail every time is $(1 - p)^n$. The probability that it will happen on the first trial and fail on the succeeding $n - 1$ trial is $p(1 - p)^{n-1}$. But the event is just as likely to happen on the second, third, etc., trials as on the first. Hence the probability that the event will happen just once in the n trials is

$$np(1 - p)^{n-1}.$$

Continuing this process, we can easily see that the probability that it will happen m times in $m + n$ trials is

$$\frac{\binom{m+n}{m}}{\binom{m+n}{m}} p^m (1-p)^n. \quad (1)$$

From (1) we can obtain directly the probability that exactly m males and n females occur in $m+n$ drawings and the expression will be

$$p = \frac{\binom{m+n}{m}}{\binom{m+n}{m}} x(1-x)^n \quad (2)$$

where x denotes males and $(1-x)$ females.

If we call the right hand member of (2) y , then

$$y = cx^m(1-x)^n$$

and this equation represents a curve with a zero ordinate when $x=0$ and $x=1$. Since the elementary area under the curve is

$$\frac{\binom{m+n}{m}}{\binom{m+n}{m}} x^m(1-x)^n dx,$$

the number of cases which occur between the two limits (a, b) is proportional to the sum of the differentials taken from a to b , or

$$\frac{\binom{m+n}{m}}{\binom{m+n}{m}} \int_a^b x^m(1-x)^n dx.$$

Also the totality of cases will be proportional to the sum of the differentials taken from 0 to 1, or

$$\frac{\binom{m+n}{m}}{\binom{m+n}{m}} \int_0^1 x^m(1-x)^n dx.$$

Hence the probability that the ratio of the males in the jar to the entire lot lies between the two limits is

$$p = \frac{\int_a^b x^m(1-x)^n dx}{\int_0^1 x^m(1-x)^n dx} \quad (3)$$

This is the well-known theorem of Bayes.¹ This equation as it

¹ See Todhunter's "History of the Theory of Probability from the Time of Pascal to that of Laplace," 1865.

stands is applicable to cases where the number of observations is small. For cases where the number of observations is large we must modify this still further.

If we suppose the two limits to be $m's \pm \theta$ where $s = m + n$, then equation (3) may be written in the following form :

$$p = \frac{\int_{m's-\theta}^{m's+\theta} x^m (1-x)^n dx}{\int_0^1 x^m (1-x)^n dx}.$$

By successive integration by parts the denominator is evaluated, giving

$$\int_0^1 x^m (1-x)^n dx = \frac{m! n!}{(m+n+1)!} \quad (4)$$

If we let $x = m/s + z$ the numerator becomes

$$\int_{-\theta}^{\theta} \left(\frac{m}{s} + z \right)^m \left(\frac{n}{s} - z \right)^n dz,$$

which is approximately

$$\frac{m^m n^n}{s^s} \int_{-\theta}^{\theta} e^{-\frac{s^2 z^2}{2mn}} dz.$$

With the above transformation the formula becomes

$$p = \frac{(s+1)!}{m! n!} \frac{m^m n^n}{s^s} \int_{-\theta}^{\theta} e^{-\frac{s^2 z^2}{2mn}} dz. \quad (5)$$

Now if we apply Stirling's formula for large numbers (4) becomes

$$\frac{(s+1)!}{m! n!} = \frac{s^s}{m^m n^n} \sqrt{2\pi mn}.$$

Therefore (5) may be written in the following form :

$$p = \sqrt{\frac{s^3}{2\pi mn}} \int_{-\theta}^{\theta} e^{-\frac{s^2 z^2}{2mn}} dz.$$

If we assume

$$\sqrt{\frac{s^3}{2mn}} z = t$$

and substitute ω for

$$\theta \sqrt{\frac{s^2}{2mn}}$$

then as a final form we have

$$p = \frac{2}{\sqrt{\pi}} \int_0^\omega e^{-t^2} dt.$$

Since the above expression is a well-known equation for the probability integral, the degree of probability for any given limits may be determined from the table. For computation we have the following relation :

$$\omega = \theta \sqrt{\frac{s^2}{2mn}} \quad \text{or} \quad \theta = \omega \sqrt{\frac{2mn}{s^2}},$$

and

$$p = \varphi(\omega).$$

As will be seen from the above relations, the degree of probability is proportional to the amount of deviation. Therefore in any given case we can either increase or decrease the value of probability by changing the value of the deviation. Thus in order to facilitate a comparison of several sets of data, it would be advantageous to fix the value of the probability and then determine the corresponding amount of the deviation. If we take 0.75 for the value of the probability, it will be certainly high enough for practical purposes. If we adopt this system, then the corresponding value of ω is fixed and is equal to 0.814. Thus we do not even need to use the table to determine the amount of deviation. If however one wishes to find any other value of the probability than I have proposed (*i. e.*, 0.75), such can be readily obtained from the table. Thus the determination of the deviation can be made by a simple arithmetical process.

The following will illustrate a method of determination. Dr. King has kindly supplied the data for this purpose and I wish to thank her for that material.

Example : Out of 16,100 tadpoles, 9,949 are examined. 5,136 are found to be females and 4,813 males. Find the probability that the ratio of females to the entire lot lies between given limits.

We have

$$\theta = \omega \sqrt{\frac{2mn}{s^2}} \quad p = \varphi(\omega) = 0.75.$$

Since we adopted the fixed value for probability (0.75) the value of ω is also fixed and equal to 0.814. Thus our problem is therefore to find a value of θ when the value of p is known.

$$\theta = 0.814 \sqrt{\frac{2 \times 4,813 \times 5,136}{99,493}} = 0.814 \times 0.00709 = 0.0058.$$

$$\text{Deviation} = 16,100 \times 0.0058 = \pm 93.$$

$$\text{Total number of females} = 16,100 \times 5,136/9,949 = 8,311 \pm 93.$$

With this value of probability we conclude that the total number of females in the entire lot is neither greater than 8,404 nor less than 8,218.

TEMPERATURE AS A FACTOR IN THE DETERMINATION OF SEX IN AMPHIBIANS.

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In 1905, Hertwig (1) published the results of a series of experiments on the eggs of *Rana temporaria* and of *Rana esculenta* which showed, according to his interpretation, that temperature is a sex-determining factor in amphibians: a high temperature favoring the development of females; a low temperature causing the production of relatively more males. This conclusion is in accord with the results of Issakówitsch's (2) earlier experiments on sex-determination in *Daphnia*; but it is opposed by the results of Maupas' (7) experiments on *Hydatina senta* and by those of von Malsen (6) on *Dinophilus apatris*. The latter experiments seemed to indicate that heat leads to the development of males and that cold tends to the production of females. Maupas and Hertwig believe that temperature acts directly on the sex-cells: Issakówitsch and von Malsen, on the other hand, maintain that nutrition is the dominant factor in sex-determination, temperature acting only indirectly through its influence on the processes of assimilation in the parent organism.

In the spring of 1907, I made a series of temperature experiments on the eggs of the toad, *Bufo lentiginosus*, which were carried out in a manner similar to those made by Hertwig on the eggs of *Rana*. Various lots of fertilized eggs were placed in tanks in which the water was kept at a nearly constant temperature until the tadpoles underwent metamorphosis (King, 5). In *Bufo* as in *Rana*, the sexes cannot be distinguished until the end of metamorphosis unless the gonads are sectioned. It is conceivable, therefore, that sex in these forms may not be determined until a relatively late period and that temperature, acting during the entire growth period of the tadpoles, might alter the normal sex-ratio. Two series of experiments were made with eggs from different females. In one series (lot A) 62.5 per cent. of the in-

dividuals in which sex was ascertained were females, although one half of the eggs had developed in water which had a temperature of 22–30° C. while the other half of the eggs had been kept at a temperature of 14–18° C. In the second series (lot B), the eggs were subjected to conditions similar to those under which the eggs of lot A had developed. Only 33.97 per cent. of females was obtained in this series, and more females were found among the individuals which had developed at a temperature of 14–18° C. than among those which had been kept at the higher temperature. The results of these experiments, therefore, do not support Hertwig's contention, and they seem to indicate that temperature, acting during the development of the tadpoles, does not determine sex in *Bufo*.

In the series of experiments described above the eggs belonging to lot A were fertilized in water which had a temperature of 16–18° C.; the eggs used for lot B, on the other hand, were fertilized in water which had a temperature of only 11–13° C. The results obtained in the experiments suggested the idea that temperature, acting at the time that the eggs were fertilized, might determine sex. This suggestion was tested in the spring of 1908 in the following way: a pair of toads were placed in water which was kept at a temperature of 26° C. until the eggs had been deposited and fertilized; the eggs from a second female were laid and normally fertilized in water which had a temperature of 9° C. There was thus a difference of 17° C. in the temperature of the water in which these two sets of eggs were fertilized. In each series as many individuals as possible were carried through to metamorphosis and their sex ascertained. The results obtained in these experiments are summarized in the following table.

TABLE I.

Water Temperature	Number Sex Ascertained	Males	Females	Per Cent Females
26° C.	2,178	953	1,225	56.24
9° C.	2,683	1,181	902	43.30

The average proportion of females among young toads that have recently completed their metamorphosis is 51.62 per cent., judging from the number of females found among the 9,949

individuals whose sex I have ascertained during the course of the experiments which I have made to study the problem of sex-determination in this form. No very definite conclusions can be drawn from the results of the experiments summarized in Table I. The percentage of females obtained from the lot of eggs that was fertilized at a temperature of 26° C., although higher than the probable average for the species, is still within the limits of possible normal variation in the proportion of females developing from eggs laid by different individuals. A relatively low percentage of females developed from the lot of eggs that was fertilized at 9° C., yet this percentage is about the same as that obtained in one of the series of experiments made to ascertain the influence of nutrition on the determination of sex in *Bufo* (King, 4). It may be possible to explain the results obtained in this last series of experiments, as well as those of the first series of temperature experiments briefly described above, as due to the fact that occasionally a batch of eggs gives an unusually low or an unusually high percentage of females and that by chance such exceptional lots of eggs were used for these experiments.

As this last series of experiments was carried out it is open to serious criticism. The eggs that were fertilized in warm water were laid by one female, while those that were fertilized in the cold water were laid by another individual. This gives an opportunity for the results to be influenced by possible normal variations in the sex-ratios of different lots of eggs. The two sets of eggs were fertilized by sperm from different males also and, as Morgan (8) has pointed out, there is the possibility that the male is responsible for the determination of sex in amphibians.

In order to avoid all obvious sources of error and, if possible, to obtain results that would give definite conclusions, another series of experiments was made this past spring with the eggs of *Bufo*. On April 7, 1909, a female which had just begun to lay was killed by pithing and the eggs removed to a dish of fresh water. Lots of about 350 eggs each were artificially fertilized in water which was kept at given temperatures (35° C., 30° C., 20° C., 10° C., 5° C.) for twenty minutes and then allowed to come gradually to the room temperature (18° C.). In all cases sperm from the same male was used. The extreme temperatures at

which lots of eggs were fertilized were 35° C. and 5° C. It is highly improbable that in a state of nature the eggs of *Bufo* are ever fertilized at these temperatures which are very injurious to the unsegmented egg, although they do not seriously interfere with the development of segmented eggs unless allowed to act for a comparatively long period of time (King, 3). Not more than one half of the eggs subjected to extreme temperatures were fertilized. Segmentation of these eggs did not begin until some three hours after the experiments were started, and in a majority of cases the cleavage planes came in very irregularly. A considerable number of eggs that began segmentation died within a few hours, so that but a very small number of individuals continued their development. After the eggs in these lots had reached the gastrulation stage they had apparently outlived the injurious effects of extreme temperature, as only three of the tadpoles that developed from the eggs that were fertilized in water with a temperature of 35° C. and but two from those that were fertilized at 5° C. died before it was possible to ascertain their sex. The great majority of eggs that were fertilized at medium temperatures, 30° C., 20° C. and 10° C., segmented normally and continued their development. The mortality in these lots was very low, not more than 6-8 per cent. of the individuals dying before it was possible to ascertain their sex. The various lots of tadpoles received similar food and were all kept as nearly as possible under similar external conditions during their entire growth period.

In this series of experiments, and also in several earlier ones, many of the tadpoles died after beginning their metamorphosis. In a considerable number of these individuals it was necessary to section the gonads in order that sex might be ascertained. The toads that survived metamorphosis were placed in large glass jars containing moist sod, and they were fed on small insects for several days. Young toads grow very rapidly, and by keeping them in the manner indicated for a short time it is possible to ascertain the sex of practically every individual without resorting to the tedious process of preserving and sectioning the gonads.

The results of this series of experiments are summarized in Table II.

TABLE II.

Water Temperature.	Number Sex Ascertained.	Males.	Females.	Per Cent. Females.
35° C.	90	42	48	53.33
30° C.	197	96	102	51.26
20° C.	323	157	166	51.39
10° C.	214	108	106	49.53
5° C.	64	33	31	48.43
Total	888	436	451	50.90

With a difference of 30° C. in the temperature of the water in which the two lots of eggs at the extremes of the series were fertilized there is a difference of only 4.95 per cent. in the proportion of females that developed in these lots. This difference is so small that it is evident that temperature, acting at the time of the fertilization of the eggs, is not the dominant factor in the determination of sex in *Bufo*.

In this series of experiments, with one exception, the proportion of females that was obtained in the different lots varied directly as the temperature at which the eggs were fertilized; relatively fewer females developing as the temperature of the water was lowered. The decrease in the percentage of females was so slight in the various cases, however, that I do not think it can have much significance, particularly as there were so few individuals in the lots at the extremes of the series. Had the number of individuals that metamorphosed been the same in every lot, practically the same sex-ratio would doubtless have been obtained for the entire series.

For the purpose of comparison, and to show more conclusively than is shown by the results of the series of experiments summarized in Table II. that temperature, acting at the time of the fertilization of the egg, does not determine sex in *Bufo*, the results obtained in former experiments in which different batches of eggs were laid in water with a known temperature are brought together in Table III.

TABLE III.

Water Temperature.	Number Sex Ascertained.	Males.	Females.	Per Cent. Females.
26° C.	2,178	953	1,125	56.24
16-18° C.	232	87	145	62.50
11-13° C.	156	103	53	33.97
9° C.	2,083	1,181	902	43.30

A comparison of the figures given in Table II. with those of Table III. shows that there is not the uniformity in the results of these various series of experiments that one would expect to find if temperature alone determined sex at the time of the fertilization of the egg. A lot of eggs that was fertilized at a temperature of 26° C. gave a higher percentage of females than was obtained from a lot that was fertilized at a temperature of 35° C., while the highest percentage of females obtained as yet in any experiment (62.5 per cent.) was found in a lot of individuals that developed from eggs that were fertilized at a temperature of 16–18° C. Of the various series of eggs that were fertilized in cold water, those that were fertilized at 9° C. gave a much lower percentage of females than did other lots that were fertilized at 10° C. or at 5° C. Although these results show very conclusively that temperature, acting at the time of the fertilization of the egg, is not the dominant factor in the determination of sex in *Bufo*, they do not exclude the possibility that temperature may have an indirect action on the determination of sex in this form. In every series of experiments lots of eggs that were fertilized at a temperature of 13° C. or below have given a lower percentage of females than has been obtained in the individuals developing from lots of eggs that were fertilized at higher temperatures. It is conceivable that a low temperature might act more injuriously on the female-producing spermatozoa than on those that are male-producing, if it be that there is a dimorphism in the spermatozoa of *Bufo* and that the male determines sex. This would, of course, greatly increase the chances that an egg laid in cold water would be fertilized by a male-producing spermatozoön. If, on the other hand, sex is determined in the egg, it is possible that the sex-determining mechanism is so evenly adjusted that temperature, under certain conditions, may turn the scale in one direction or the other.

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DIFFERENTIATION IN HYDROID COLONIES. II. AGLAOPHENIA.

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In a former paper¹ I called attention to the fact that many species of hydroids change their structural type with age, that is, in the direction of their growth. "Stems straight proximally may become sinuous distally. Branches which alternate during the early stages of colonial development may later originate in pairs. Length and annulation of hydrothecal pedicels, size, proportions and ornamentation of hydrothecæ may similarly vary with the distance from the base of stem or branch." An attempt to analyze the problems in differentiation suggested by these facts was begun, upon a species of campanularian peculiarly well adapted for experimentation. The results reached favored the view that the progressive changes in the differentiation of the individuals of a colony are not dependent upon changes of external conditions, but upon changes of internal conditions; and that these changes are of a physiological character, and do not lead to a conception of *morphological determinants* or *residual germ plasm*.

The bearing of the facts upon the problem of senescence was also suggested. For, on the stem of such a hydroid as *Campanularia*—with a cymose type of budding—the individuals appear as successive generations, forming, at the same time, parts of the whole colony. Accordingly, a comparison of any two individuals might be expected to show a difference in their structure corresponding in some degree to a period of physiological change in the colony equal to their difference in age.

Since the publication of these results, several important papers have appeared expressing radically different views on the relation of natural death and development. Minot (*Pop. Sc. Mo.*, Nov., 1907, p. 472) holds that as cells differentiate they lose something of their capacity to live. Loeb (*Pflüger's Arch.*, 124, 1908, p. 411), after stating this conclusion in chemical terms as follows:

¹Univ. Cal. Publ., Zoology, 2, p. 323.

that the chemical causes of death are identical with the chemical processes that lie at the basis of development itself, differs with it radically. He asserts, as a result of experiments with the eggs of sea urchins, that natural death and development are determined by chemical processes that have absolutely nothing to do with each other.

My own investigations, impeded, among other causes, by scarcity of material, have not yet reached a definite conclusion in this direction, although the results so far obtained are not inconsistent with the results of Loeb's brilliant experiments. Meanwhile, the facts as they appear in seven species of *Aglaophenia*, may be of some service to experimenters interested in the general problem.

As is very well known, a typical colony of the plumularian hydroid *Aglaophenia* closely resembles a feather, of which the shaft is represented by the stem and the vanes by two ranks of alternating branchlets, or hydrocladia, corresponding to barbs. Each hydrocladium is divided by more or less definite nodes into internodes and bears on one aspect — the same in all hydrocladia — a compact series of hydranths, one to each internode, with tooth-rimmed hydrothecæ. Associated with each hydrotheca are three tubular nematophores, a pair laterally placed, to the rear, and one in front in the median line (mesial).

Inspecting such a colony for signs of the differentiation connected so clearly with age in *C. bakeri*¹ one would naturally look for differences associated, first, with the growth of the colony as a whole, second, with the growth of individual hydrocladia. The former might be determined by a comparison of corresponding internodes of different hydrocladia, the latter by a comparison of successive internodes of the same hydrocladium.

In both cases, this procedure has established a correlation between differentiation and growth.² Since the picture of the correlation is not equally clear and elaborate in all species studied, I will describe it first for one of the most satisfactory in these respects, namely, *A. octocarpa*.

¹ *Loc. cit.*

² See Pearl, Publ. Carnegie Inst. 58, 1907, and, more recently, Ritter, Univ. Cal. Publ. Zool., 6, 1909, p. 64.

It should be understood, in examining all the tables and figures which follow in this paper, that they make no pretense to be

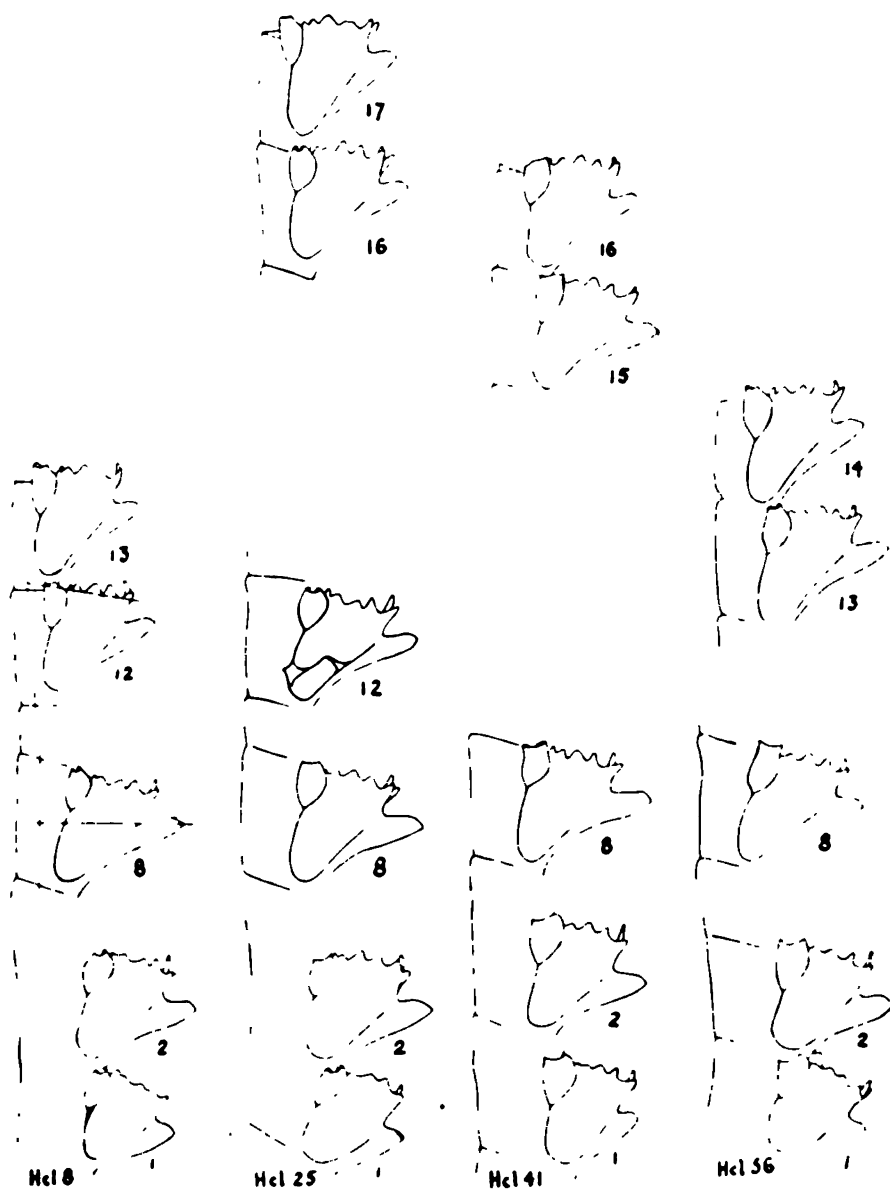


FIG. 1. *A. olearia*, = 45.

statistically thorough. They are rather illustrative of certain facts of differentiation that do exist and are capable of experimental analysis.

A. octocarpa. — A glance at the accompanying camera drawings (Fig. 1) will acquaint one with the main differentiating characters on which the present study is based. The four series of figures represent hydrothecæ from four hydrocladia. That they might be representative of the colony, the latter were selected at various levels on the right of the stem, being nos. 8, 25, 41 and 56, in a series of 64,¹ and care was taken that they should be the longest obtainable and, if possible, not damaged at their distal ends. Similarly, the hydrothecæ, drawn in profile, were chosen as representative of the whole hydrocladium, being nos. 1, 2, 8, usually 12, and the terminal two.

In the condensed picture of the conditions throughout the colony thus obtained, certain facts stand out prominently:

1. Each hydrocladium tapers from base to tip.²
2. On each hydrocladium, hydrotheca no. 1 is noticeably smaller than the others, and the mesial nematophore associated with it is especially short.
3. From base to tip, the openings of the hydrothecæ become less oblique to the axis of the hydrocladium. This obliqueness may be shown numerically by measuring the angle made with the axis of the internode by a line drawn tangent to the depressions between the first and second, and the third and fourth, lateral marginal teeth on each hydrotheca (12, *Hcl.* 8, Fig. 1). Two series of measurements from different colonies are shown in Tables I. and II.
4. The mesial nematophore reaches its greatest prominence toward the middle of the latter and then declines to the tip. This fact is numerically expressed in Table III., which shows the profile width of each hydrotheca to the point of the mesial nematophore on a line perpendicular to the axis of the internode (see Fig. 1, *hydrocl.* 8, *lth.* 8).

¹ The tip of the colony was wanting.

² This, as shown by repeated observations, is independent of the thickening of the perisarc with age. For convenience, neither such thickenings of the walls nor the septal and intrathecal ridges characteristic of the species have been drawn, except in 12, *Hcl.* 25, where the intrathecal ridge is shown.

TABLE I.
Measurements in Degrees of Angle.

Hydrocladium.	Hydrotheca.								
	1	2	8	12	13	14	15	16	17
8	114	110.3	108.2	103	96				
25	103.8	99.3	108	102.8				100	96
41	115.2	109	105				108	99	
56	116.2	116.5	105.5		99	91.2			

TABLE II.

Hydrocladium.	Hydrotheca				
	1	2	7	11	
1	122	111.5	101.5	89.7	(12 hydrothecae present)
40	99.7	111.8	101.5	100	(14 " " ")
85	111.3	111.2	103.7	100.5	(13 " " ")

TABLE III.
Measurements in mm. $\times 95$.

Hydrocladium.	Hydrotheca.								
	1	2	8	12	13	14	15	16	17
8	24	30.5	32	27	25.5				
25	25	32	34	31				29.5	27.5
41	23.5	30	33.5				31.5	29	
56	25.5	30.5	30.5		30.5	27			

The facts formulated above concern the differentiation of internodes of single hydrocladia from various regions of the colony. On further examination, Tables I. and III. suggest that the character under consideration tends to decline from its maximum less rapidly on hydrocladia in the middle region of the colony than on hydrocladia either proximal or distal to them. This suggestion that the colony may have a typical form, exhibiting a differentiation from base to tip, analogous to what is so familiar in the leaves of plants, is supported by series of measurements at the tips of several colonies. One such series is presented in Table IV., in which are given both the length of each theca, through the unpaired or mesial tooth (upper figures), and the width of the theca through the tip of the mesial nematophore (lower figures in italics), as in Table III.

TABLE IV.

Measurements in mm. $\times 95$.

Hydrocladium.	Hydrotheca.																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
3 (from tip)	—	36.5	38	38													
	27	32	32	31													
4 " "	—	37	37.5	37	37												
		33	34.5	33	31												
7 " "	—	37	—	38.5	38.5	38	38	—	38	36							
		33	—	36	35	35	34	—	33	31							
10 " "	—	—	37	—	38	—	38	—	38.5	—	38	38					
			34	—	35	—	35	—	35	—	31.5	28					
13 " "	32	35.5	38	38	38.5	—	37	—	38	38	38	38	37.5	38.5	37	38	—
	25±	34	35	35	34	—	35	—	35	35	34.5	34.5	34.5	34	33	30.5	—

It will be seen that, as the tip of the colony is approached, not only do the hydrocladia possess fewer and fewer hydrothecæ, but the dimension of the latter through the mesial nematophore reaches its minimum more and more rapidly. Since the hydrothecæ, once formed, do not enlarge with age, it is clear that for such colonies as this, there is a limit of growth and a specific form.¹

A. pluma. — This species not only supports the statements made for *A. octocarpa*, though somewhat less conspicuously, but presents another character (of no value in *A. octocarpa*) by which differentiation in the hydrocladia may be determined, namely, the length of the hydrotheca through the mesial tooth. Fig. 2 presents graphically, in concentrated form, the characters of a single colony so far as they concern the discussion. The measurements taken from the drawings are represented in the accompanying tables. It should be said, in this connection, that these measurements are subject to a certain amount of correction, owing to the great difficulty experienced at times in obtaining true profiles of the hydrothecæ. In hydrocladium no. 25, for instance, the hydrothecæ have suffered a degree of rotation which could not be rectified by the means at my disposal. The application of pressure sometimes suffices, but is very liable to produce more serious distortions. Fortunately, the errors which

¹ According to Table IV., the length of the hydrothecæ does not appear to be a significant dimension in *A. octocarpa*.

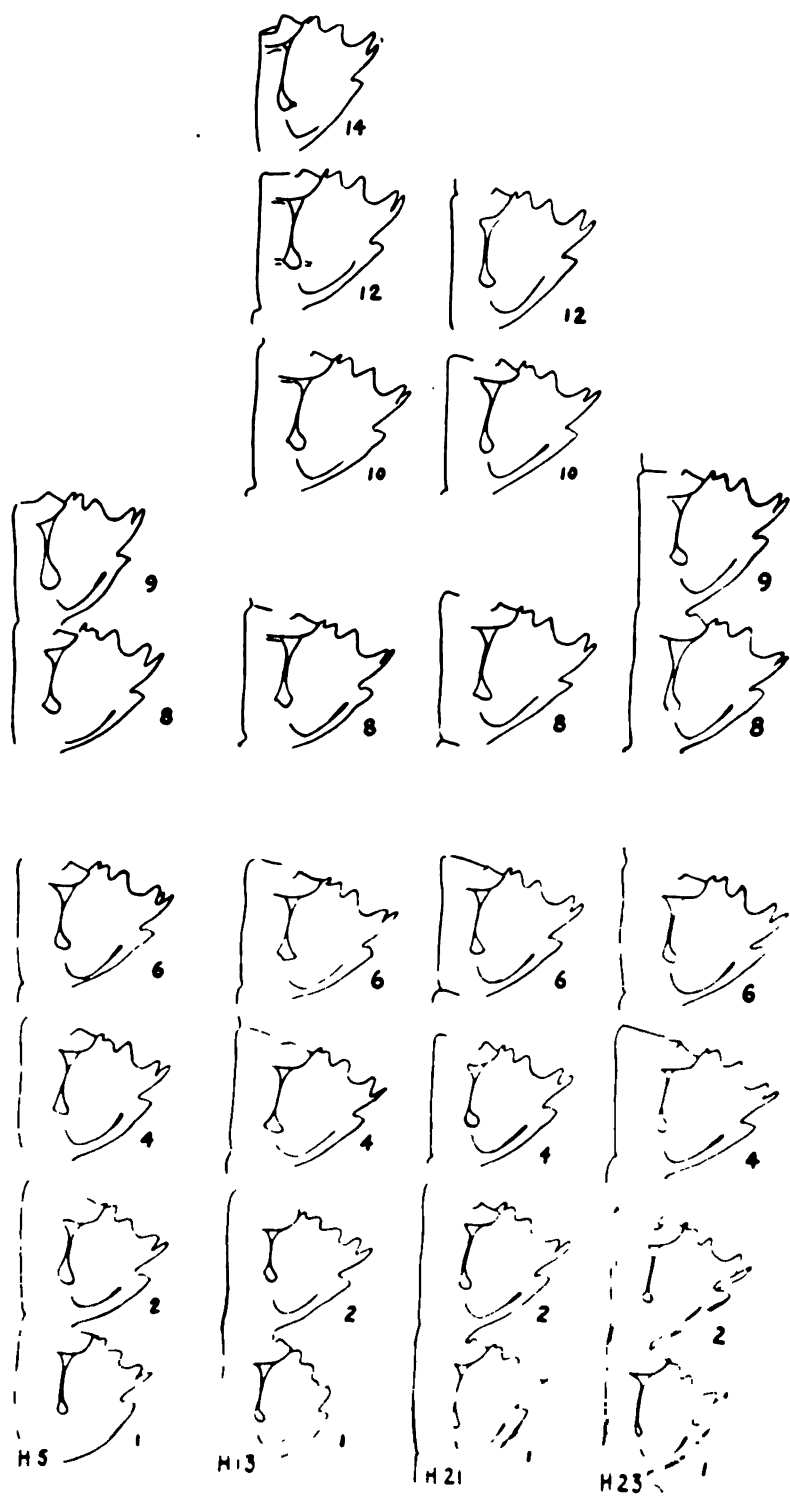


FIG. 2. *A. puma*, - 45.

thus appear to be unavoidable, do not disturb in any essential respect the conclusions based on the measurements.

Differentiation within the several hydrocladia will first be considered.

In Table V., appear the measurements of the length of the mesial nematophore along a line perpendicular to the axis of the internode (upper figures) and the width of the hydrotheca to the tip of the nematophore along the same line (lower figures). The width of the hydrotheca along the line chosen varies, as shown by the table, independently of the length of the nematophore along the same line. Neither character is of much value

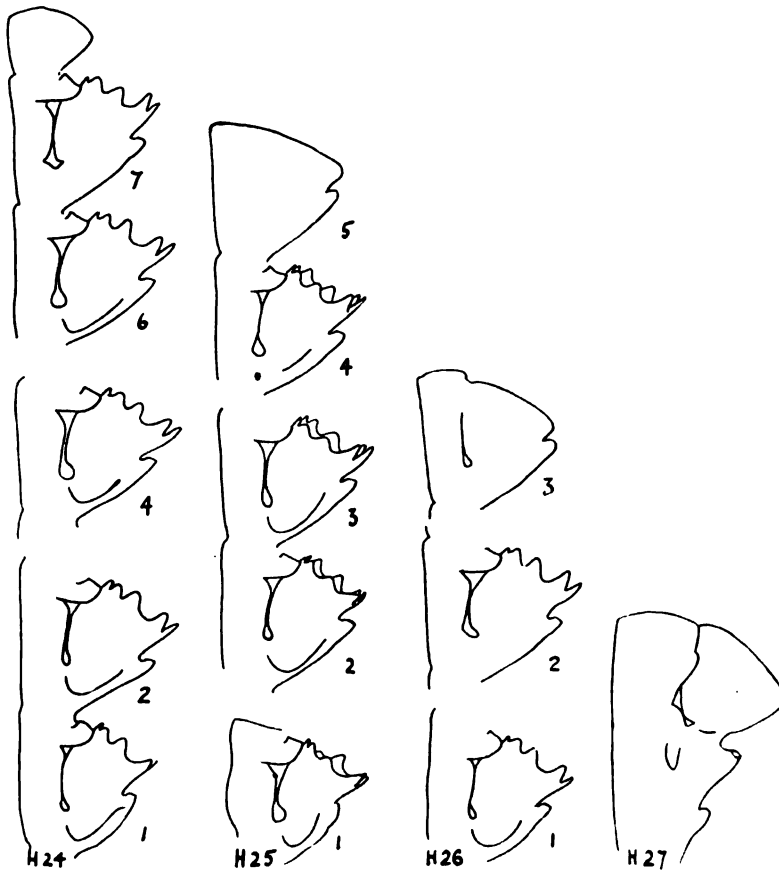


FIG. 2

to the experimenter in this species, owing largely to the slight degree to which the nematophore projects, and the narrow range of its variation in length, in fractions of a millimeter, as compared with the fluctuations in width of the hydrothecæ. There is no doubt, however, that the nematophore tends to vary, as in *A. octocarpa*, with the growth of the hydrocladium, its maximum length lying in the middle region of the latter.

TABLE V.

Measurements in mm. $\times 95$.

Hydrocladium.	Hydrotheca.													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
27	bud													
26	3.3 21.3	3.5 25	bud											
25		4.5 23.5	4 24.5	4 23	bud									
24	3.4 10.3	4.2 23.5		4.6 24.5		4 25.5	3.8 24.5	bud						
23	3.8 19.5	3.8 22		4.5 25.5		4.4 26.5	3.5 24.5	3.8 24.5	bud					
21	3.8 19.7	3.2 23		3.5 23		4 23.2	3.5 24.5		3 24.5			3.8 24.5	bud	
13	2.7 18.5	3 21.5		3 23.5		3.5 24	3.9 24		3.6 23.5			3.2 24.5		2.8 21.5
5	3.3 19.5	4 22.5		3.5 24		4.7 25	4 25	3 21.5						

The obliqueness of the openings of the hydrothecæ is recorded in Table VI., in degrees of angular distance, as for *A. octocarpa*, with this exception, that the line defining the obliqueness is drawn tangent to the depressions between the mesial and first lateral, and the third and fourth laterals (the margin having but nine teeth). This is a more stable standard, but could not be utilized in *A. octocarpa*, owing to the shape of the hydrotheca in that species.

With slight irregularities, the chief among them being connected with the variability of hydrotheca no. 1, the obliqueness diminishes toward the distal end of each hydrocladium.

TABLE VI.
Measurements in Degrees of Angle.

Hydro-cladium.	Hydrotheca.													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
27	bud													
26	128	125.5	bud											
25	128	131.5	125.5	126	bud									
24	129.8	130		128.5		123.3	119.3	bud						
23	127.5	129.8		129.3		125.5		123.5	119.3	bud				
21	130.3	129		120		120		120		119		115.3	bud	
13	—	125		128.2		123		124		121.5		117		115
5	133	131		121.5		121.5		120.2	115					

The lengths of the hydrothecæ, taken through the mesial tooth, are recorded in Table VII. There is a definite tendency to increase in length toward the distal end of each hydrocladium, the longest hydrotheca, however, being subterminal.

TABLE VII.
Measurements in mm. $\times 95$.

Hydrocladium.	Hydrotheca.													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
27	bud													
26	30.5	?	bud											
25	27.5	31.5	34.5	?	bud									
24	30.5	33.5		34.2		36.5	bud							
23	28	30.5		34.5		36		35	36.5	bud				
21	31	33		32		33		35+	36		35	bud		
13	—	30		31.5		34.5		34		36		37.3		32.5
5	—	29.5		33		34.5		35.5	33.5					

In Table VIII., the widths of the hydrothecæ previously used have been taken along the line defining the obliqueness of the opening of the hydrothecæ. As the figures show, this character is too variable to be very useful to the experimenter. Nevertheless, in common with the characters considered in the three tables

TABLE VIII.

Measurements in mm. $\times 95$.

Hydrocladium.	Hydrotheca.													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
27	bud													
26	20	22	bud											
25	18	18.7	20.5	20	bud									
24	17.5	21		21.5		21	20	bud						
23	19	21.2		21		22		21.5	21	bud				
21	17	20.5		20		21.5		22.7	21		22	bud		
13		20.5		20.5		20.5		20.5	21.2		22.7		20.5	
5	17.5	20		20.2		22		22	19.7					

just preceding, it aids in establishing the fact that colonies of *A. pluma*, like colonies of *A. octocarpa*, have a limited growth and a specific form, the hydrocladia, with respect to given characters, differing in different regions. To appreciate this fact, it is only necessary to note the position (1) of the maxima on the various hydrocladia in Tables V. (upper figures), VII. and VIII., and (2) of the minima in Table VI., especially in connection with hydrocladia 5, 13, 21, 23 and 24.

A. inconspicua.¹—This small species is represented most scantily in the collections of the University of California. No conclusions can be offered concerning the form of the colony as a whole, as observations on the tip of a mature colony are at present out of the question. From the drawings (Fig. 3) of the internodes from two hydrocladia, however, and the measurements based upon them, Tables IX. and X., the species possesses admirable characters for experimental work, in the prominence of the mesial nematophore and the obliqueness of the hydrothecal opening.

These figures are typical, similar measurements from other hydrocladia and other colonies giving results that vary, within the hydrocladium, in no essential respect from them. It may be said that the hydrotheca at the base of each hydrocladium is especially variable and often differs conspicuously from those distal to it in size, shape, marginal dentation, and position on the

¹Torrey, Univ. Cal. Publ., Zool., 1, 1902; *ibid.*, 2, 1904.

hydrocladium (owing to a slight rotation out of the plane of the other hydrothecæ). Nothing sufficiently definite can be said about the length or thickness of the hydrothecæ. There is a perceptible increase in size of the hydrothecæ in *Hcl. 21*, toward the tip. It is much less in the other hydrocladium; and meas-

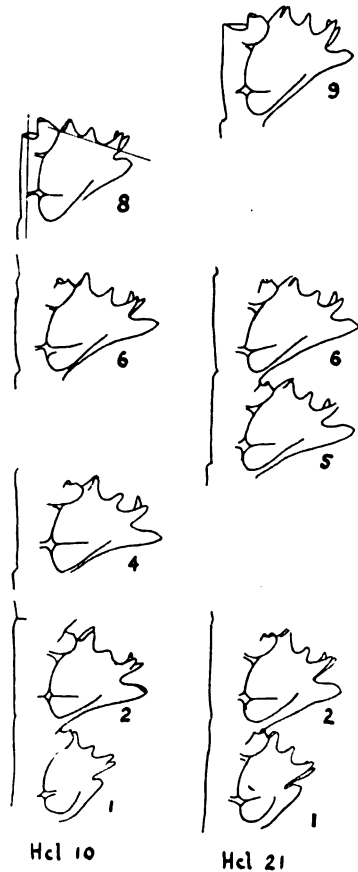


FIG. 3. *A. inconspicua*, $\times 45$.

urements of others show that it is not characteristic and typical, for either dimension.

A. lophocarpa. — The few colonies of this species at hand are all imperfect, both as to stems and hydrocladia. However, the hydrocladia taper from base to tip, the first hydrotheca on each

TABLE IX.

COLONY WITH 28 HYDROCLADIA.

Measurements, in mm. $\times 95$, of the profile width of each hydrotheca in Fig. 73 to the point of (1) the mesial nematophore on a line perpendicular to the axis of the internode (lower figures), and (2) the length of the mesial nematophore on the same line (upper figures).

Hydrocladium.	Hydrotheca.						
	1	2	4	5	6	8	9
10	2.5 16	7.3 26	8.2 29.5		8 24	4.8 24	
21	4.5 17	7 26		8.8 28.5	7.6 29		7.5 27.5

TABLE X.

Same colony, showing angles made with the axes of their internodes by the planes of the hydrothecal openings, the latter being represented in each case by a line tangent to the depressions between lateral marginal teeth 1 and 2, and 4 and 5 (see *Hcl. 10*, *Atk. 8*).

Hydrocladium.	Hydrotheca.						
	1	2	4	5	6	8	9
10	125.5	121.3	122		114	105	
21	132.3	130		123.2	121		105.3

is smaller than the others, and there is a distinct tendency toward a reduction of the obliqueness of the hydrothecal opening, from base to tip. The mesial nematophore, also, projects farther from the hydrotheca in the intermediate internodes than at either extreme of the hydrocladia.

A. struthionides. — The obliqueness of the hydrothecal openings in each hydrocladium is practically constant in this species (Fig. 4). As in *A. octocarpa*, however, each hydrocladium tapers from base to tip, and the mesial nematophore is most prominent between the extremes. The prominence of the nematophore is, perhaps, of all the characters, most useful to the experimenter. The measurements in Table XI. show that the length of the nematophore along a line passing through its tip and perpendicular to the axis of the internode, varies independently of the width

of the hydrotheca along the same line from the base of the nematophore (see Fig. 4, *BHcl*, *hth.12*).

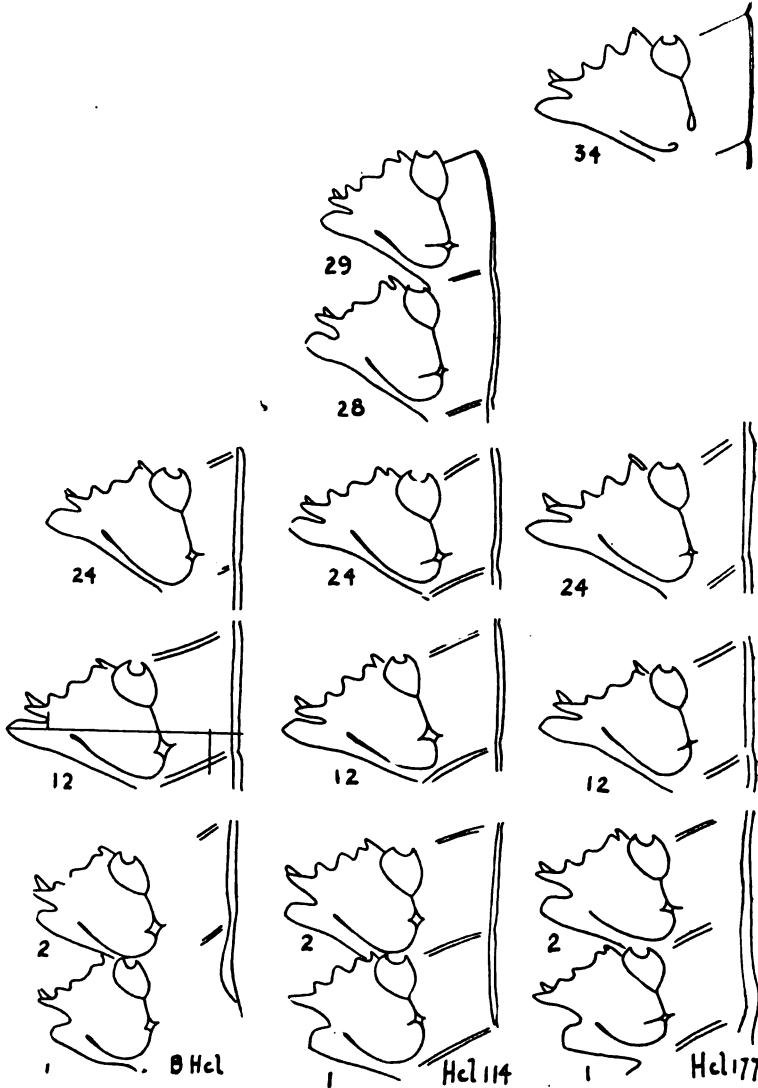


FIG. 4. *A. struthionides*, $\times 45$.

Neither this nor the following species lends itself easily to a determination of colonial differentiation, as does *A. pluma*. Both are variable, often luxuriant growers. Colonies of *A.*

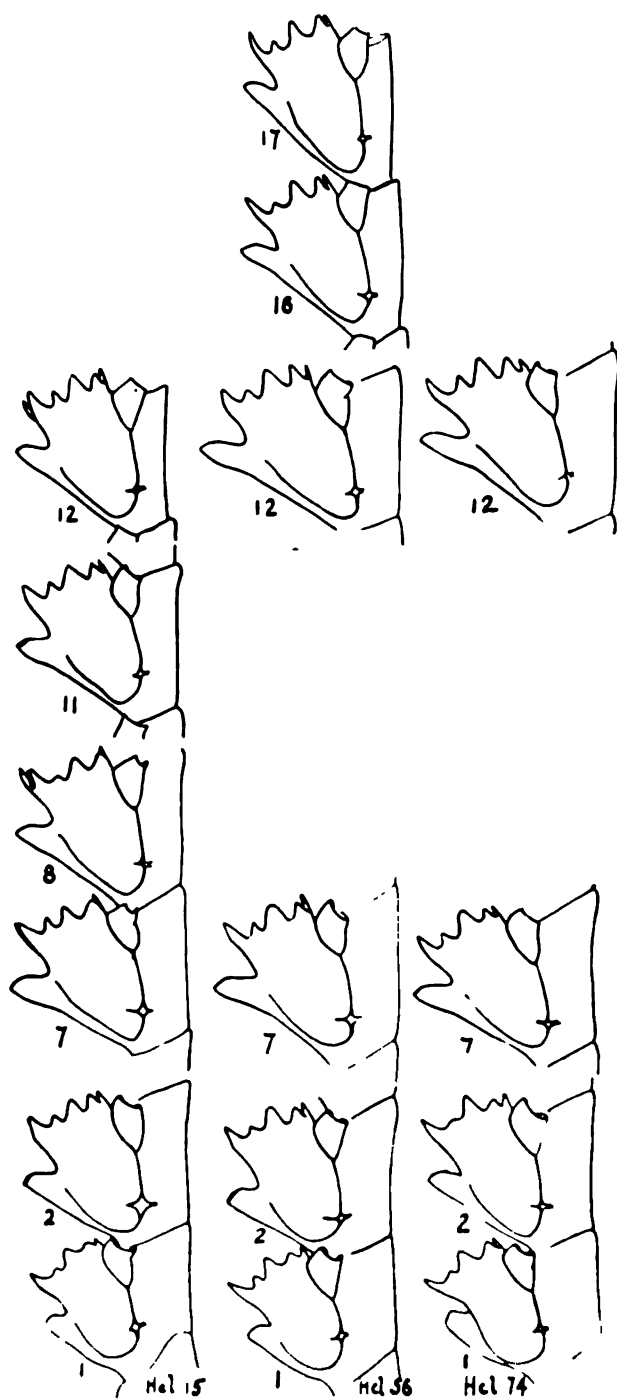


FIG. 5. *A. la. phemia* sp., 45.

TABLE XI.
Measurements in mm. $\times 95$.

			Hydrotheca.			
			2	12	24	34
<i>Hcl.</i> at base	1. Base of nem. to tip		5	11	9	
	2. Base of nem. to rear of theca		26.4	29.5	28.5	
	3. Value of fraction $1 \div 2$		.19	.37	.31	
" 114	1.		8.2	11.3	9.9	
	2.		27	28.7	28.6	
	3.		.30	.39	.35	
" 177	1.		8.2	11.3	12	9.5
	2.		24	27.8	30.7	32.3
	3.		.34	.40	.39	.29

struthionides may exceed 23 cm. in length. *Aglaophenia* sp. has a particularly stringy, sprawling habit. The character is of no practical value in the present connection.

Aglaophenia sp. — This species differs from *A. struthionides* in no particulars essential to the present discussion.

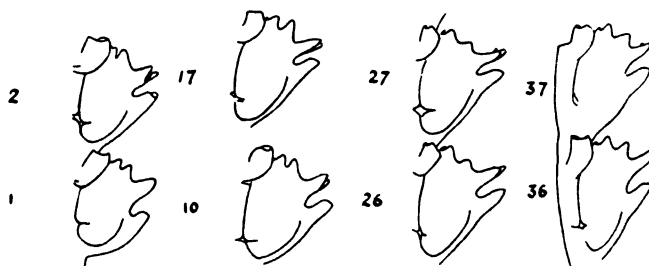


FIG. 6. *A. diegensis*, $\times 45$.

TABLE XII.

Distance from tip of nematophore to rear of theca along perpendicular to axis of internode. Measurements in mm. $\times 95$.

Hydrocladium.	Hydrotheca.							
	1	2	7	8	11	12	16	17
15	24	31	34.5	33	32	31		
56	24.5	30	35.5			40	32	30
74	25	29.5	34.5			37.5		

Hydrocladium 74 is incomplete, hydrotheca 12 not being terminal.

A. diegensis. — Of all the species, this is the most irregular. It agrees in general, however, with *A. octocarpa*. The hydrocladia taper distalward; the first hydrotheca is small and more or less irregular, with short mesial nematophore; the mesial nematophore is most prominent between the extremes of the hydrocladia; and there is a slight lessening of the obliqueness of the hydrothecal openings distalward. Fig. 6 is from a single hydrocladium.

ZOOLOGICAL LABORATORY,
UNIVERSITY OF CALIFORNIA,
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BIOLOGICAL BULLETIN

THE SEAT OF SMELL IN THE CRAYFISH.

S. J. HOLMES AND E. S. HOMUTH.

The location of the sense of smell in the crustacea has been the subject of but few investigations. Putnam, Graber, Bateson, Dearborn, Spaulding and others have made observations on the reactions of various decapods to food at a distance from the body, although they were not primarily concerned in tracing the seat of the olfactory reactions. Herrick, working on the lobster, found that most parts of the body were sensitive to ammonia and clam juice. Experiments of Nagel¹ and Bethe² indicate that, in the decapods, the first antennæ have an important olfactory function, although both these investigators regard the olfactory sense organs as not exclusively located in these appendages. Nagel worked with *Pagurus*, *Astacus* and *Carcinus*. Removal of the antennules of *Pagurus* was followed by reactions to chemical stimuli, although these were not so pronounced as before the operation. *Astacus* in the presence of food was found to show lively movements of the antennules, followed by movements of the mouth parts and legs. *Carcinus* showed the same movements of the mouth parts and antennæ in the presence of food, but it was only when food came into actual contact with the mouth parts that efforts were made to secure it. Nagel's experiments led him to conclude that sight and touch play a greater part in locating food than the sense of smell.

Bethe found that if a piece of meat is thrown into a dish with several *Carcinus* there is no reaction at first. After several seconds the antennules begin to wave rapidly and the maxillipeds move slowly back and forth. The animals nearest the piece of meat

¹ Bibliotheca Zoologica, Bd. 18, 1894.

² Archiv f. mik. Anat., Bd. 50, 1897.

give this reaction first. The farther the *Carcinus* is from the meat the later will begin the reactions. Soon after the animals begin to react they move about and usually in quite a direct line toward the meat. The nearer they approach the food the faster they go. That the eye plays little part is evidenced by the fact that blinded forms go toward the meat with just as much certainty. If a piece of meat is passed over the rocks at the bottom of the aquarium a *Carcinus* will follow this scent and often pick up every stone, which has been in contact with the meat, and pass it to the mouth, but as soon as the stones come in contact with the maxillipeds they are rejected.

If the antennules are removed *Carcinus* reacts to food but only when it is placed a short distance from the mouth parts. The latter are regarded by Bethe as also sensitive to chemical stimuli. In the amphipod *Amphithoe*¹ one of the present writers found that the antennules were important olfactory organs, but that there remained a certain but much diminished power to detect food at a distance from the mouth parts after the antennules, or even the antennules and the antennæ were removed. Other species of amphipods were found to make exploring movements of the antennules when in the presence of food.

Nagel's experiments with amphipods and isopods failed to elicit any positive response, although some species gave negative responses to disagreeable or irritating substances. The terrestrial isopods, *Oniscus*, *Porcellio* and *Armadillo* he found to be quite insensitive to strong substances like cedar oil and benzol. It is not difficult, however, to obtain very decided reactions from terrestrial isopods to the vapor of acetic acid and ammonia even after removal of the antennæ.

Many papers have appeared dealing with the organs of smell in crustacea but conclusions regarding their functions have been based more on morphological grounds than on the results of experiment. One of the most recent papers on the chemical sense of crustacea is that of Bell,² who worked on the reactions of the crayfish. Bell applied meat juice by means of a fine-pointed pipette, to various parts of the body. To render it evi-

¹ BIOLOGICAL BULLETIN, Vol. 2, p. 165, 1901.

² *Jour. Comp. Neur. and Psych.*, 10, 299, 1900.

dent when the substance came in contact with the animal, it was colored with eosin or carmine. It was found that stimulation of various parts of the body would elicit a response. The animal moved its mouth parts, became restless, moved the small chelipeds about and sooner or later attempted to go toward the source of stimulus. The responses varied somewhat according to the region to which the stimulus was applied, but there was a marked tendency, as we have also found, for the first reaction to manifest itself by a movement of the part most directly stimulated. The antennules, antennæ, mouth parts and tips of the large and small chelipeds were found to be organs of especial sensitiveness. We have repeated Bell's experiments with meat juice and verified in the main his results. Bell, however, did not test his conclusions by removal of certain organs or by operations on the nervous system. One is never quite sure that by using chemicals which diffuse through the water only the organ is stimulated to which the stimulus is applied. It was thought desirable, not only for this reason, but on account of determining more definitely the role of certain organs in chemoreception to observe the reactions of crayfish after the removal of the antennules and antennæ, and after the destruction of the brain or the division of the nerve cord.

The outer ramus of the antennules bears, in addition to the kinds of setæ found on the antennæ and other parts of the body, certain peculiar club-shaped organs which have generally been considered the end organs of the sense of smell. These organs are absent on the inner ramus, and on the antennæ as well as other parts of the body. Organs of a similar character are common in other crustacea, being in many cases more abundantly developed in the male. This circumstance, as well as the fact that removal of the antennules diminishes the response to olfactory stimuli, renders it quite probable that these club-shaped organs perform the function which has so often been assigned to them. In a series of experiments we have compared the reactions of crayfish with the outer ramus of the antennules removed with the reactions of normal animals and individuals otherwise operated on. The rami were removed several days before the animals were experimented with in order to eliminate any effect of shock

on the reactions. All of the specimens were kept for the same period without food, were placed in the same kind of dishes and treated in all respects as nearly as possible in the same way. Only individuals which had been resting quietly for some minutes were employed for experiment. If any reaction was produced by the movements of the observer the crayfish was not used for experiment until it had been left for a considerable time and became perfectly quiet. A piece of meat was very carefully introduced into the water with a long fine forceps and held a quarter of an inch above the rostrum. If one's movements are exceedingly slow it is possible to work around crayfish even when recently brought in from the field without exciting alarm. The number of seconds was noted before any of the feeding responses of the legs or mouth parts were set up. Sometimes such movements appear to arise spontaneously, and therefore only those cases were counted in which these movements eventuated in seizing and devouring the meat. In three sets of experiments performed at different times the time reactions of the crayfish were as follows :

EXPERIMENT I.

	Time of Reactions in Seconds									Average.
Outer ramus removed	60	80	42	16	36	220	64			74
Inner ramus removed	80	34	64	74	30	6				48
Both rami removed	110	170	270	88	180	156	58	116		131
Normal animal	46	12	6	30	36	68	30	24		31

EXPERIMENT II.

Outer ramus removed	184	148	92	272						174
Inner ramus removed	36	20	24							27
Both rami removed	50	64	120	90						81
Normal animal	2	18	6							8.7

EXPERIMENT III.

Outer ramus removed	10	24	26	24	44					25.6
Inner ramus removed	10	18	24	42						23
Both rami removed	112	120	412	26						167.5
Normal animal	26	16	26							22.7

EXPERIMENT IV.

(Specimens with eyes blackened over.)

Outer ramus removed	72	116	180	120	36	94	30	110	144		100	
Inner ramus removed	6	12	4	4	4	6	36	12	4	48	14	13.7
Both rami removed		42	112	242	270							141.5

EXPERIMENT V.

(Specimens with eyes blackened over.)

Outer ramus removed	74	68	214	56	168															118
Inner ramus removed	10	42	14	32	102	32	34	27	6	33										33.2

The experiments show in a striking enough way that removal of the outer branch of the antennules is followed by a much greater loss of sensitiveness to olfactory stimuli than is caused by removal of the inner ramus. But they also show that crayfish with the inner ramus removed react less promptly than do normal animals. We may conclude from these experiments that the part most sensitive to olfactory stimuli is the outer ramus of the antennules, but that the inner ramus is also to a certain extent sensitive to the same kind of stimuli. Specimens with both rami removed respond to meat juice or pieces of meat placed near the tip of the large second antennæ. It is scarcely possible that any chemical could diffuse to other parts of the body before the reaction takes place and hence the second antennæ must have an olfactory function also. Specimens in which both antennules and antennæ were removed showed a marked power of responding to bits of meat or meat juice placed near the mouth parts or the tips of the chelæ. The small chelipeds were found especially sensitive to substances placed near the tip. By very carefully applying the tip of a pipette which had been drawn out into a long slender tube near one of the small chelæ and slowly forcing out a little meat juice, one usually sees a slight grasping movement of the chelæ, often at first a small twitch of the dactyl, followed by movements of reaching about. These movements are followed by exploring movements of the other chelipeds, chewing movements of the mouth parts, and by a turning of the body toward the stimulus.

Animals in which the brain was destroyed were also experimented with. The crayfish was securely fastened to facilitate working upon it. With a fine-pointed scalpel a piece of the carapace about one fourth of an inch square was cut out just over the brain. After destroying the brain the piece of the carapace was carefully replaced and the edges sealed with asphalt varnish. The animal was then kept out of water until the asphalt had dried and the wound sealed. After a few days the animal was experi-

mented with and showed marked responses from the mouth parts and chelipeds. When the chelipeds were rubbed with a piece of filter paper the substance was seized but rejected if passed to the mouth parts. When meat was pressed against the chelipeds in the same way it was seized more eagerly, produced a greater degree of excitement and was generally eaten. To make sure that this was not due to a difference in the consistency of the two articles two wads of filter paper were taken upon one of which was pressed some meat juice. The two wads were applied to the chelipeds in the same way, but the one with the meat juice was more quickly seized and was generally passed to the mouth parts and swallowed. In some instances the pure filter paper was swallowed, but this was very rare.

The chelipeds were also stimulated simultaneously, the one with pure filter paper, the other with filter paper soaked in meat juice. The animal almost invariably turned toward the side with the meat juice and grasped more vigorously on that side. The stimuli were frequently transposed but this did not affect the result; the reaction to the paper with the meat juice was the more vigorous.

In one crayfish the nerve cord was cut across directly behind the mouth parts and in front of the large chelipeds. The result of this operation is to somewhat paralyze the animal, especially the anterior chelipeds which are drawn up under the body. Objects that touch the chelæ are frequently passed to the mouth but again rejected. A bit of meat, however, is chewed and swallowed although this act requires a much longer time than in the normal animal. In some cases the chewing movements ceased before the food was swallowed. The chelipeds when stimulated by meat grasp it more eagerly than they seize innutritious materials, and the animal in general shows a greater degree of excitement.

The experiments recorded show that the outer ramus of the antennules which bear the so-called olfactory setæ are especially sensitive to olfactory stimuli; that the inner ramus of the antennules and the antennæ are also sensitive to olfactory stimuli but to a less degree; and that the olfactory sense is developed in other parts of the body, especially the mouth parts and tips of the chelipeds.

ANALYSIS OF FORM REGULATION WITH THE AID OF ANÆSTHETICS.

C. M. CHILD.

During the last year and a half the writer has carried on an extensive series of experiments on the influence of certain anæsthetics on the course and results of form regulation in *Planaria dorocephala* Woodworth. Since the method has proved to be of considerable value and since it will be some time before the results of these and other experiments planned for the future can be presented in full, it seems desirable to present briefly and without extended comment the most important features of the work up to date as an illustration of the value of the method and the results to be attained by it.

In my experiments thus far I have used chiefly alcohol, ether and acetone-chloroform, known commercially as chloretone. Although alcohol is much less strongly anæsthetic than many other substances its action is in general similar in kind to that of the substances commonly known as anæsthetics. The results with the three substances mentioned are practically identical so far as the more general features are concerned, though various differences of detail appear, which need not be considered here. Incidentally, however, it may be noted that these differences in the effects of the different anæsthetics serve to some extent as a means of determining the specific differences in the effects of different anæsthetics on the metabolic processes: how far they will prove to be of value in this direction only the future can determine.

In my experiments various concentrations of the three substances have been employed, but the most characteristic results in general have been obtained with alcohol 1.5 per cent., ether 0.4–0.5 per cent., chloretone 0.025–0.0375 per cent. These concentrations cause a considerable deathrate, but the results in the pieces which do not die within the first week or ten days are very uniform, so far as pieces of the same size and from the same region of the body are concerned. Alcohol has been used to a

larger extent than either chloretone or ether in my experiments up to the present time because it has given characteristic results and because it has seemed desirable to obtain fairly complete data concerning the effect of a mild anæsthetic upon various regions of the body, on pieces of different size and age and in different physiological condition as a basis for comparative work with substances with stronger anæsthetic properties. It is my intention to extend the work to various other groups of substances which have a definite effect on metabolism. In most of the work of this kind the effect of only a short period of time in the given medium has been determined. It seems to me particularly desirable to determine more exactly the effects of continued existence in media of various constitution on development and regulation. By such means it should be possible to advance a step further in our analysis of the processes of differentiation, localization and growth.

I. METHODS.

The method of use of the anæsthetics, all three of which are more or less volatile, is as follows : Not more than ten pieces of the planarian are placed in a Stender dish holding about 250 c.c. and with ground edge and cover with ground groove exactly fitting the edge. This dish is filled to three fourths of its depth with the mixture of the desired concentration, some of the fluid is poured over the under side of the cover so as to wet its surface and fill the groove, and the cover is placed in position at once. Several of these dishes are placed in larger glass jars with ground edges, a liter or more of the fluid is poured over them into the jar and it is at once sealed by means of a glass plate on which a ring of vaseline corresponding to the edge of the jar has been smeared. In this way decrease in concentration is avoided as far as possible. Furthermore the fluid is renewed at least every forty-eight hours and the mixture of the desired concentration is always made up immediately before using. It has been found desirable to keep the temperature as nearly as possible constant, except where its influence on the results was to be determined. In most of my experiments the temperature has ranged between 17° and 20° C. but rarely beyond these limits. Rise in temperature much beyond 20° C. increases the

death-rate and in temperatures below 15° C. the regulatory processes are considerably retarded and certain complicating features which result directly from low temperature appear. With fairly constant temperature and regular renewal of the fluid it has been possible to keep various individuals and pieces alive in 1.5 per cent. alcohol for several months, and death, when it occurred, was apparently the result of starvation. As will appear below, however, the length of life of the pieces and individuals varies greatly according to conditions.

II. CERTAIN FEATURES OF THE ORGANIZATION OF PLANARIA.

In order to make clear certain of the results presented below it is necessary to call attention to the fact that in *Planaria dorotocephala* (and in *P. maculata*) the posterior region of the body in all animals above a certain small size is specified to a greater or less extent as a second individual or zooid, which, when it attains a certain degree of physiological independence, separates from the other part by an independent motor reaction and becomes a complete animal. As I pointed out some years ago,¹ the presence of this second zooid is indicated by the regional differences in the regulatory power of short pieces taken in sequence. When we compare pieces not more than one tenth the length of the whole animal and as nearly as possible of equal length, we find that the ability to form a head decreases in each successive piece from the original head-region posteriorly, until at about the region of the old pharynx the power to form a head disappears entirely and the pieces remain headless. But at about the middle of the post-pharyngeal region, *i. e.*, at the level where fission occurs, the power of head-formation reappears rather suddenly. In species like *Planaria simplicissima*, which do not undergo fission, there is no increase in the power of head-formation in the postpharyngeal region. Other regional differences which indicate the presence of a second zooid in the posterior region of the body are described in the paper referred to above. As further evidence along the same line, I may add that I have been able to induce fission experimentally without feeding under conditions where it does

¹ Child, "The Relation between Regulation and Fission in *Planaria*," BIOL. BULL., XI., 3, 1906.

not occur normally, *e. g.*, in animals reduced by starvation from a length of 15–18 mm. to a length of 6–7 mm., in young animals of 7–8 mm. in length, in the pieces resulting from the fission of large individuals, and again in the pieces resulting from the fission of pieces derived from fission.

It follows from these facts that the posterior end of the first zoöid lies somewhere near the middle of the postpharyngeal region in large individuals, and posterior to this level is a second young individual.

III. THE GENERAL EFFECTS OF THE ANÆSTHETICS.

In the first place the regulatory processes are much retarded and the retarding effects are greater in small than in large pieces. The most favorable cases in the anæsthetics require at ordinary temperatures ten days or more to reach a stage which similar pieces in water attain in four or five days.

In a short paper¹ attention has already been called to the fact that the change in the proportions of the pieces is retarded to a much greater extent than the formation of new parts. As a matter of fact the increase in length and the decrease in width of the pieces, which is so characteristic a feature of the regulation in *Planaria* does not occur to any appreciable extent until the pieces have become sufficiently acclimated and have reached a sufficiently advanced stage of development to move about in a manner approaching the normal. In the paper above referred to I pointed out that this fact confirms the conclusions which I have already stated in previous papers, *viz.*, that this change in proportion is largely the result of mechanical conditions connected with movement and locomotion.

In general the formation of the new tail is retarded to a much greater extent than that of the head. A small amount of new tissue forms on the posterior cut surface, but outgrowth to form the typical, tapering posterior end does not occur until the specimens begin to creep about. Apparently the movement and use of the posterior end is not only a factor in determining its shape, but also stimulates the growth of new tissue.

¹ Child, "The Regulatory Change of Shape in *Planaria dorotcephala*," *Biol. Bull.*, XVI., 6, 1909

The process of regulation ceases in the anæsthetic before the degree of development attained in water is reached. The head remains of smaller size in proportion to the size of the piece, the "auricles" on the sides of the head, which in this species are normally long and pointed, remain short and blunt, the new pharynx attains only a fraction of the size attained in water, and intestinal regulation, with the exception of the degeneration of the smaller intestinal branches in many cases, does not occur to any appreciable extent until the animals begin to move about actively.

These results are sufficient to show that the method affords a means of separating more or less completely in time certain regulatory processes which under normal conditions occur together, and thus of determining in some degree their relations to each other, and in the cases of change of shape and development of the tail their relation to movement and use of parts.

Another most interesting difference between the pieces in anæsthetics and those in water is the relation between regeneration in the stricter sense and redifferentiation. In certain pieces, which in water form the whole head back as far as the eyes by regeneration of new tissue, only the more anterior portions of the head are formed in this way in anæsthetics. In this respect alcohol and ether differ to some extent: in ether apparently less regeneration and more redifferentiation than in alcohol occurs, and in some cases in ether only the extreme tip of the head is regenerated, all other parts being the result of redifferentiation. A case of this kind is figured in my paper referred to above (9, Figs. 14-16). As regards this point the process of regulation resembles that occurring after long continued starvation, but the substitution of redifferentiation for regeneration is even more complete in the anæsthetics than after starvation.

IV. THE EFFECT IN RELATION TO THE SIZE OF THE PIECE.

In general a larger piece is necessary for the production of a whole, or anything approaching a whole, than in water. Within certain limits the death-rate increases as the size of the piece decreases. Regulation is retarded to a much greater extent in smaller than in larger pieces. In smaller pieces the formation

of a single median eye-spot instead of two, bilaterally symmetrical in position, is much more frequent than in larger pieces. With decrease in size all stages between the fully developed head — *i. e.*, as fully developed as it ever becomes in anæsthetic media — and headless pieces can be obtained.

All of these differences in regulation which are connected with differences in size can be observed in water, but in the anæsthetics they occur in much larger pieces than in water.

V. THE EFFECT IN RELATION TO REGION OF THE BODY.

The regional differences are similar in character to those observed in water but some of them are more marked. In the anæsthetics, for example, pieces from the middle region of the body, even those as long as one fourth of the whole length, almost always remain headless, while in water only very short pieces from the middle region fail to produce heads. Pieces one fourth the length of the body taken from the region immediately behind the old head almost always produce heads in the anæsthetic as well as in water. In short, it is almost impossible to inhibit head-formation in pieces from this region, though there is not the slightest difficulty in inhibiting it completely in pieces of the same size from the middle region. When we compare pieces of the same size taken in sequence from the anterior end posteriorly, we find that the ability to form a head disappears much more rapidly in anæsthetics than in water, but at the region of fission it appears again in both media and the pieces from the posterior region of the body, *i. e.*, the pieces of the second zoöid, so far as they survive — they are like pieces of younger animals in being more sensitive than pieces from other regions when first placed in the anæsthetic, and in becoming more rapidly and completely acclimated — produce a larger percentage of normal heads (that is, normal for the medium) than pieces from any other region.

Manifestly the comparison of pieces from different regions shows much more clearly than any series of experiments in water that the ability to form certain parts is present in very different degree in different regions of the body, and since the effect of the anæsthetics is to depress the metabolic processes or certain of them, the ability to form certain parts is apparently closely connected with the intensity of certain metabolic processes.

VI. THE EFFECT ON LOCALIZATION AND NUMBER OF PARTS.

The localization of the pharynx in anæsthetics is different from its localization in water. In prepharyngeal pieces the pharynx appears nearer the posterior end and in postpharyngeal pieces, nearer the anterior end than in water. This difference in the localization of the pharynx means that the process of redifferentiation does not extend so far from the posterior end in prepharyngeal pieces, or from the anterior end in postpharyngeal pieces as in water. Moreover, the shifting of the pharynx toward the middle, which occurs in each piece in water, is greatly retarded and in many cases never completed in the anæsthetics.

This change in the localization of a specific organ resulting from a change in the external medium is of considerable interest and has a bearing upon the problem of localization in general. Driesch has maintained that the localization of morphogenetic processes in "harmonious equipotential systems," of which he regards *Planaria* as an example, cannot be accounted for on a physico-chemical basis. In this case the facts certainly demonstrate that a change in the constitution of the medium, of such a character as to affect the metabolic processes, brings about a change in the localization of a specific organ, the pharynx.

Another case which involves not merely localization but number of parts as well, concerns the eye-spots. Normally two eye-spots, symmetrically placed, appear in *Planaria* in regulation as well as in ontogeny. As noted above, however, we find that short pieces in water, especially those from the middle region of the body, often give rise to only one median eye-spot. In fact the formation of a single eye-spot instead of two is the first indication of decreased or incomplete ability to form a head. If we compare pieces of different lengths with anterior ends at the same level of the body we find that with decreasing length of piece single eye-spots appear more frequently, until in pieces of a certain length perhaps only single eye-spots are formed. With still further decrease in the length of the pieces the heads with single eye-spots give way to a headless condition. It should be noted, however, that the relation between the length of the piece and the character of the result is by no means constant, but is dependent on the length of the animal, the physiological condition

and various other factors. In shorter individuals, for example, a relatively larger fraction, though an absolutely shorter piece, is necessary for a certain result similar to that produced by a relatively smaller fraction, though absolutely longer piece from a longer individual. Moreover, even in the same individual paired eyes appear in pieces of a certain length from the anterior region and the region of the second zoöid, while pieces of the same length from the middle region produce either single eyes or else remain headless. With continued starvation the length of a piece necessary to produce a given result as regards eyes increases.

With this brief statement of the results of experiments in water we may now turn to the consideration of the anæsthetics. In the anæsthetics single eyes appear in much longer pieces than in water. By preparing similar pieces of the proper length from a certain region of the body and placing part of them in alcohol, or ether, part in water, it is often possible to obtain 100 per cent. of single eyes in the anæsthetic medium and 100 per cent. of normal paired eyes in the water.

But in the anæsthetic a rather remarkable further change occurs very frequently, though I have never observed it with certainty in water. After some two weeks or more in the anæsthetic the single-eyed heads give rise to two additional eyes symmetrically placed in the normal position and slightly posterior to the single median eye already present. These three-eyed individuals are a characteristic feature of the experiments with anæsthetics. All the eye-spots persist as long as the animals live, whether they remain in the anæsthetic or are returned to water.

But still another feature must be noted. The longer pieces in the anæsthetic frequently produce two eyes in the normal position. In many cases such pieces give rise after two weeks or more to a second pair of eye-spots a short distance behind the first pair and, like them, symmetrically placed. Such individuals then possess four eye-spots, all of which persist during life.

Briefly then a very common effect of the anæsthetic is to increase the number of the eye-spots beyond the normal. It should perhaps be noted that these additional eye-spots are not mere fragments of pigment, but typical eyes, the pigment being

associated with the unpigmented area representing the sensory cells in each case. Frequently, however, in the four-eyed individuals the unpigmented areas as seen in dorsal view in the living animals are continuous between the anterior and posterior eye on each side. But the pigment spots are, so far as my observations go, always distinct and arise separately, not by division of a pigment mass already present. An attempt to consider the possible factors involved in these peculiar phenomena must be postponed until the data are presented in full. It may be said, however, that these cases of supplementary eye-formation suggest that the formation of a single eye or of a pair under normal conditions inhibits the formation of further eyes within a certain region of the head, while in anæsthetic media this correlative inhibiting effect is not sufficient to prevent the formation of new eyes as the animal becomes more and more fully acclimated and the head grows larger.

The relation between the formation of single median eyes or paired eyes and the length of the piece in anæsthetics and in water demonstrate very clearly that even the localization of such an organ as the eye-spot is dependent, not merely on the character of the tissues from which it is formed, but upon the organization of the whole piece of which it forms a part. Moreover, the fact that, other things being equal, a longer piece is necessary for the formation of paired eyes in the anæsthetic than in water indicates that the conditions or processes in other parts of the piece are less effective in the localization of the eyes in anæsthetic media than in water.

VII. THE EFFECT ON THE WHOLE ANIMAL.

When whole animals of large size (15–18 mm.) are placed in the anæsthetics the first effect besides the more or less complete cessation of locomotion is usually the loss of the pharynx, which is often extruded within forty-eight hours, though it may be retained and undergo degeneration in situ. Following its extrusion or degeneration a small new pharynx is usually slowly formed in the old pharyngeal pouch, but this never attains anything like the size of the original organ. Small young animals usually do not extrude the pharynx, and, so far as I have been able to determine, it does not degenerate when retained.

The heads of whole worms in anæsthetics gradually assume the shape characteristic of heads regenerated under the same conditions. The auricles decrease in length until scarcely visible, the head becomes smaller in proportion to the body and its outlines become more rounded.

In many cases, though by no means always, the large individuals begin after a few days in the anæsthetics to degenerate in the region representing the posterior end of the first zoöid, *i. e.*, the anterior half of the postpharyngeal region, and this degeneration results in the complete separation of a posterior portion corresponding to the second zoöid and an anterior portion consisting of the first zoöid minus more or less of its posterior end. In some cases the disintegration proceeds gradually in each piece after separation until the whole is disintegrated, but very commonly it ceases after the separation of the two parts, the surfaces heal and the posterior piece develops a new head as it would do after normal fission, while the anterior piece produces a small amount of new tissue at its posterior end. This process of disintegration is not due to an infection or to any other accidental condition in the medium, as is clearly shown by two facts: first, it increases in frequency with decreasing temperature. At low temperature practically every large individual separates into two parts while in room temperatures separation often occurs in less than half. And second, such disintegration and separation almost never occurs in individuals below a certain size, either in high or low temperatures. In short we find that the posterior region of the first zoöid in large individuals is subject to degeneration and disintegration in anæsthetics, and more frequently in low than in high temperatures, while in small individuals degeneration rarely or never occurs in this region.

I can account for these facts only as follows: In the large individual the first zoöid is approaching the limit of size and its posterior end being most distant from the centers of correlation, the cephalic ganglia, is approaching a condition of what we may call physiological isolation. When we place such individuals in the anæsthetics we decrease the effective distance of transmission of the nervous processes and the posterior region of the first zoöid becomes still further isolated. This region is incapable of form-

ing a new animal in anæsthetics, as we can readily show by isolating it physically, *i. e.*, by cutting it out and placing it in an anæsthetic medium, where it almost always degenerates. Consequently its condition while still attached to the other parts is comparable to its condition when physically isolated, *i. e.*, it is then physiologically isolated to such a degree that it behaves as if physically isolated and disintegrates. When we add the effect of low temperature to that of the anæsthetic, the physiological isolation is more certainly and more completely induced, consequently disintegration of this region is more frequent in low temperatures. This process of disintegration does not occur in small individuals because they are far below the limit of size and the posterior end of the first zoöid is not physiologically isolated in anæsthetics to any such degree as in the larger individuals.

VIII. ACCLIMATIZATION.

If the individuals or pieces live for a week or more in the anæsthetic they became more or less acclimated and begin to move about and react in the usual manner, though very slowly and imperfectly. In many cases they continue to live for months, especially in alcohol. In ether and chloretone I have not as yet been able to keep them alive for so long a time in concentrations corresponding in their effect on regulation to those of alcohol employed for this purpose. By beginning with mixtures of low concentration and gradually increasing the concentration they can be acclimated to concentrations which otherwise kill them within a few hours. I have not thus far attempted to determine the limit of acclimatization, since my attention has been directed chiefly to other problems. It is of interest, however, to note that young animals become more readily and more completely acclimated to mixtures of given concentration than do older ones, although the younger individuals show a higher death-rate than the older when first placed in the anæsthetic. And finally individuals which have been reduced in size by starvation do not show the same power of acclimatization as young animals of the same size, but on the contrary, their ability to become acclimated decreases as starvation and the decrease in size continue. These facts bear upon various recent attempts at

interpretation of the reduction processes as reversals of development (Lillie, Schultz and others). It is evident from my experiments that the individuals reduced by starvation, although they may be simpler morphologically, than the full-grown individuals, are physiologically not younger but older than these.

IX. LENGTH OF LIFE.

In an extended series of experiments I have attempted to collect data concerning the length of life in anæsthetics of constant concentration, with the result that some conclusions of interest have been reached.

As noted above, young individuals of small size are more sensitive than older, larger, and die in greater numbers during the first two or three days, but if they survive this period they very commonly live longer than the larger older animals, since they become more completely acclimated. Pieces from the posterior region of the body of large animals and corresponding to the second zoöid behave in the anæsthetics like young animals.

Animals and pieces which have been well fed up to the beginning of the experiment live longer in anæsthetics than starving individuals and pieces. It is of interest to note that the further starvation advances, the earlier do the animals die in anæsthetics of given concentration.

Pieces from different regions of the body show very different degrees of resistance to the anæsthetics. Pieces from the anterior region without the head live much longer than pieces of the same length from the middle region. Short pieces including the old head die much earlier than longer pieces and their death is not due to reduction in consequence of starvation to the limit of existence, for they die long before such reduction occurs. In other series of experiments pieces have been allowed to begin the process of regulation in water and have been placed in the anæsthetic at various stages. If we compare long and short pieces with anterior ends at the same level of the body, we find in general that, as regulation proceeds, the short pieces, which have undergone a more extensive reorganization than the long pieces, behave more and more like young animals when placed in the anæsthetic, *i. e.*, they are more sensitive at first, but if they survive they become more

readily and more completely acclimated than the longer pieces and outlive them. In short the more extensive the regulatory reorganization of the piece the younger it is physiologically, and vice versa. These facts bear upon the general problems of age and "rejuvenation."

X. CONCLUSION.

The above brief statement of results includes only the more important features of the experiments, but is, I think, sufficient to demonstrate the value of the method and the possibility of its application to various problems. I hope to extend the experiments with these and other substances and with other species, in order to obtain a broader basis for comparison. I scarcely need call attention to the possibilities of the method for the analysis of morphogenetic phenomena.

The results attained by anæsthetics can be approached more or less closely and in certain respects by decreasing the supply of oxygen, by low temperature and by starvation. In this respect my experiments are in accord with the hypothesis suggested by various authorities, viz., that anæsthetics inhibit to a greater or less extent the fundamental metabolic reactions or certain of them. We shall probably find, however, on further experiment that different anæsthetics act differently in certain respects as regards the morphogenic processes.

HULL ZOÖLOGICAL LABORATORY.
UNIVERSITY OF CHICAGO,
December, 1909.

THE CHROMOSOMES OF *ACHOLLA MULTISPINOSA*.¹

FERNANDUS PAYNE.

In a recent paper entitled "Some New Types of Chromosome Distribution and their Relation to Sex," I described in part the history of the chromosomes in *Acholla multispinosa*. At that time, I had but little material and was unable to say with certainty just what occurred in the second maturation division. However, I stated that all the evidence pointed to the conclusion that the ten chromosomes in the ring divided equally while the members of the hexad group in the middle remained undivided, five of them passing to one pole, and one to the other. I also stated that the number of chromosomes and their size relations in the male and female somatic cells made it almost conclusive how the members of the hexad group separated; that no other manner of distribution could give an end result of 26 chromosomes for the male and 30 for the female.

In order to clear up the doubtful points, I collected new material in larger quantities during the past summer and fortunately have been able to follow the complete history of the chromosomes in the spermatogonial, oögonial and the first and second maturation divisions. As a result, I can state that my former observations were entirely correct and that my inference in regard to the separation of the chromosomes in the second division has proven true.

The oögonial divisions (Fig. 1, *A*, *B*, *C* and *D*) show 30 chromosomes, 24 of which are approximately the same size while six are much smaller. I have examined female material from New Jersey, New York, Indiana and Illinois and they all show the same number and size relations. The spermatogonial groups (Fig. 1, *E*, *F*, *G* and *H*), on the other hand, contain 26 chromosomes, 22 medium sized, one very large and three small. Since the number and size relations of the chromosomes in the male and female groups are so different, it might be argued that I have been working with two species. In regard to this question, I

¹Contribution from the Zoological Laboratory of Indiana University, No. 111.

wish to state that with this point in mind, Van Duzee has examined my material very carefully and that the larger part of it (30 specimens) consisting of both males and females was collected within a small circular area of about 200 feet in diameter.

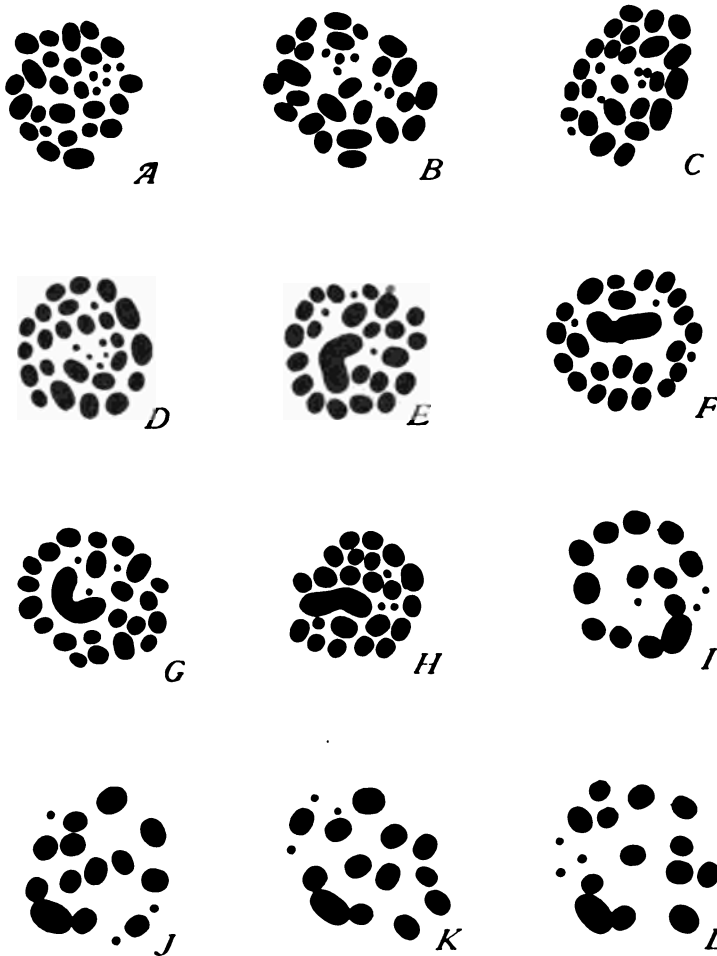


FIG. 1.¹ *Acholla multispinosa*. A, B, C and D, metaphase figures of the oögonial division, showing 30 chromosomes; E, F, G and H, spermatogonial divisions showing 26 chromosomes; I, J, K and L, metaphase figures of the first maturation division showing 16 chromosomes.

The metaphase plate of the first spermatocyte-division shows 16 chromosomes (Fig. 1, I, J, K and L). As 26 is the spermato-

¹ All figures are drawn to the same scale and magnified 1,500 diameters.

gonial number, ten of these must be bivalent and six univalent. The three small ones are again present. The large one is here as in the prophase of the first division linked end to end with two other chromosomes. All divide equally in this division. I was fortunate enough to find a side view of the anaphase showing all three small ones dividing (Fig. 2, *D*). The large one and the two

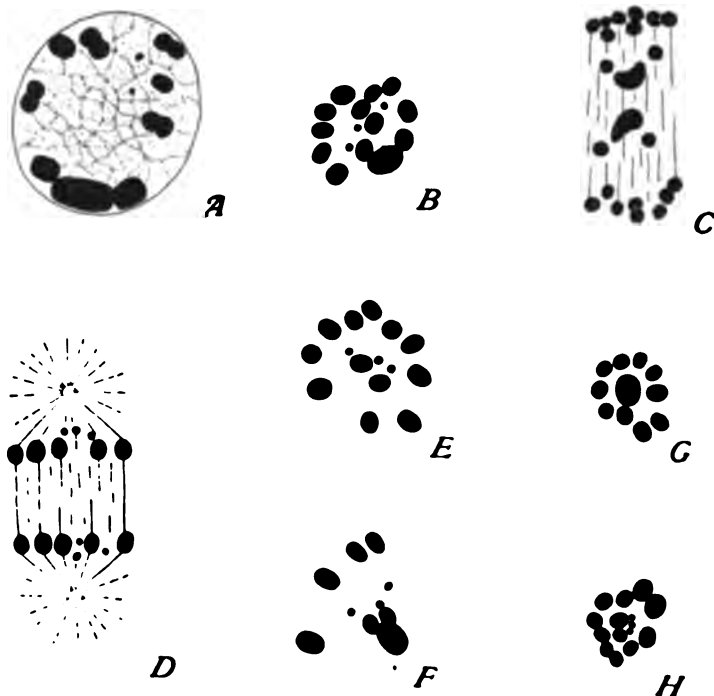


FIG. 2. *Acholla multispinosa*. *A*, prophase of the first division; the large chromosome is joined end to end with two others; *B*, anaphase, polar view, of the first division; *C*, anaphase, side view, of the first division, showing the late division of the large chromosome and the two with which it is linked; *D*, anaphase, side view of the first division, showing the division of the three small chromosomes; *E*, metaphase of the second division, polar view, showing the ten chromosomes in the ring and the five members of the hexad group which lie in one plane; *F*, a slightly oblique view of the second division metaphase showing the hexad group; *G* and *H*, anaphases, polar view, of the second division, showing the chromatin content of the two classes of spermatozoa.

with which it is joined are usually the last to divide (Fig. 2, *C*, a side view of the first division anaphase). Fig. 2, *B*, is a polar view showing 16 chromosomes. There is no definite arrange-

ment in this division, although the large one always lies on the periphery.

As a result of the equal division of all the chromosomes in the first division, the metaphase plate of the second maturation division shows 16 chromosomes. In this division, however, there is always a definite arrangement. Ten of the 16 form a more or less regular ring while the remaining six, two medium sized, three small and the extra large one, are arranged in a hexad group in the middle. Five members of the hexad group, the two medium sized and the three smaller ones, lie in one plane, while the sixth member, the large chromosome, lies either above or below the five on the other side of the equatorial plane. Fig. 2, *F*, is a view of the second division metaphase, slightly to one side, showing the arrangement of the hexad group. Fig. 2, *E*, shows the ten chromosomes in the ring and the five in the middle. The large one could not be shown without displacing it. The ten chromosomes in the ring divide equally while the members of the hexad group do not divide, but five of them, the two medium sized and the three small ones, pass to one pole and the large one to the opposite pole. The anaphases showing this unequal distribution are shown in Fig. 2, *G* and *H*. Two classes of spermatozoa are thus produced, differing in that one class contains 15 chromosomes, the other 11. Further, since the oögonial number is 30 and the spermatogonial 26, the reduced number of chromosomes in the egg must be 15 and the two classes of spermatozoa must be respectively male and female producing.

$$\text{Spermatozoön } 15 + \text{egg } 15 = 30 (\text{♀})$$

$$\text{Spermatozoön } 11 + \text{egg } 15 = 26 (\text{♂})$$

As stated in the previous account, the size relations of the chromosomes serve as an aid in reaching the above conclusions. If the fifteen-chromosome class of spermatozoa meets an egg with 15 chromosomes, three of which are small, the offspring should have 30 chromosomes, six of which should be small. This is what we find in the female cells. If the eleven-chromosome class of spermatozoa meets the same egg, the cells of the embryo should have 26 chromosomes, three of which should be small and one extra large. This condition is fulfilled in the male cells.

I have not been able to follow the history of the differential chromosomes during the growth period. A plasmosome is present. Sometimes it stains perfectly black but again it may stain much as the cytoplasm does. If the latter, a number of chromatin bodies can be seen embedded in it. As I have followed the history of the plasmosome and its relation to the differential chromosomes in *Prionidus* it seems very probable that here, too, in *Acholla* the differential chromosomes are embedded in the plasmosome during the growth period.

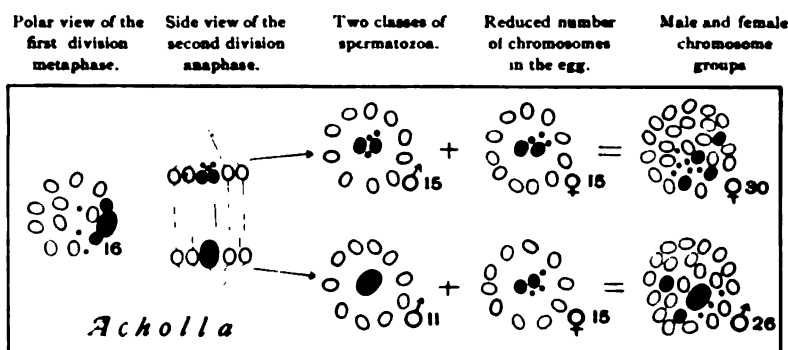


FIG. 3.

Fig. 3 gives a diagrammatic representation of what occurs in maturation and fertilization.

Only a few years ago maturation meant a reduction to one half; the number of oögonial and spermatogonial chromosomes were thought to be the same in each species and this number an even one. Even as late as 1900, Wilson in "The Cell in Development and Inheritance" writes as follows: "Van Beneden's epoch-making discovery that the nuclei of the conjugating germ-cells contain each one half the number of chromosomes characteristic of the body-cells has now been extended to so many plants and animals that it may probably be regarded as a universal law of development" Those who believe that the odd chromosome is merely a delusion in the minds of a few investigators still cling to the universality of Van Beneden's law. However, the law is no longer of universal application. Not only the odd chromosome but a number of other irregularities have been recently described,

the present case of *Acholla* giving the greatest variation in number. In fact so many variations have been found that we may justly ask what is to be the limit of these irregularities and where will they lead us?

The case of *Acholla* is interesting for two principal reasons. First, it gives us the greatest variation in number so far discovered, there being a difference of four between the male and female groups. Secondly, if we examine the size relations carefully it will be noticed that the large chromosome which goes to the male-producing pole seems to contain a larger amount of chromatin than the five chromosomes of the hexad group which go to the female-producing pole. A number of measurements show this to be the case. In all the irregularities hitherto described, the female cells contain the larger number of chromosomes and also the greater quantity of chromatin. The present case is not an exception to the number but seems to be in regard to the quantitative relations.

Several recent theories of sex determination have been based upon the quantitative relation of the chromatin. The evidence in *Acholla* forms one of the stumbling blocks in the way of some of these interpretations. To be sure, if we should ignore the large chromosome, the homologue of the small idiochromosome, or what Wilson terms the *Y* element, many difficulties would disappear. Most certainly though, as Morgan says, there is no reason for disregarding it except that its presence, in an active condition, does not fit in with our hypothesis. In his recent paper on "Sex Determination in Phylloxerans and Aphids," Morgan while realizing the difficulties in its way, further develops the quantitative hypothesis and holds to it as a rough approximation to a solution. In regard to the case of *Acholla* he suggests that possibly the five chromosomes of the hexad group which go to the female-producing pole are more active than the large chromosome which goes to the male-producing pole. The suggestion may be true, but is the question of the activity of the chromosomes the same as that of the quantitative relations?

THE POLE DISC OF CHRYSOMELID EGGS.

H. L. WIEMAN,

UNIVERSITY OF CINCINNATI.

Hegner (09b)¹ has described a disc-shaped mass of darkly staining granules at the posterior end of freshly laid eggs of two genera of beetles, *Calligrapha* and *Leptinotarsa*. In earlier publications (Hegner, 08, 09a)² these granules are spoken of as "germ cell determinants" in the sense that they fix the character of the sex cells. The use of the word determinant is open to criticism inasmuch as the term implies the attribute of certain potentialities that these granules have not been shown to possess. In the complete account of the early history of the germ cells (Hegner, 09b)¹ the word *determinant* does not appear, and the conclusion regarding their significance is summed up in the statement, that the "pole-disc" is "intimately associated with the development of the pole cells" (p. 288).

In view of the experiments of Lyon, Lillie, Morgan and others, which center about the question of the rôle of preformed materials in the egg as *versus* a predetermined method of action as the essential factor in embryonic development, it is important to know something of the nature and origin of the granules of the pole disc. The data derived from the experiments of these investigators tend to indicate that early developmental phenomena can take place even though the original configuration of the ground substance be radically changed. The formative processes do not depend on the materials displaced by centrifuging, but are bound up in the organization of the ground substance of the egg protoplasm.

Hegner did not determine the origin of the pole disc, but is inclined to believe that it is composed of particles of chromatin derived from the nucleus. No expulsion of material from the nucleus was actually observed; the conclusion being based

¹ *Journal of Morphology*, XX., 2, 1909.

² *BIOLOGICAL BULLETIN*, XVI., 1, 1908; *Journ. Exp. Zool.*, VI., 4, 1909.

mainly on staining reactions and the results of Blochmann (86),¹ Stuhlmann (86),² and others who have described in various species of hymenoptera a budding of the nucleus of the ovocyte resulting in the formation of many small "nuclei" (Nebenkerne) each containing small dark staining granules. The work of Sylvestri (08)³ is also cited as supporting a nuclear origin of the pole disc.

In connection with some work embodied in a paper now in press, I have had occasion to study the process of nutrition in the egg of *Leptinotarsa signaticollis*, a chrysomelid beetle closely related to *L. decemlineata*, one of the species studied by Hegner. In this form the nutritive material consists of a granular mass secreted by the nurse cells of the ovariole. This material enters the egg through the egg-string which is a protoplasmic process of the egg terminating in the groove-like spaces between the nurse cells. In safranin-lichtgrün preparations these granules stain as intensely with the basic dye as the granules of the pole disc.

Fig. 1 is a semi-diagrammatic drawing representing a longitudinal section through an ovary containing developing eggs. The eggs which in an earlier stage are massed together in the proximal region of the terminal chamber of the ovariole, gradually become arranged in a linear series in the ovariole stalk, the cells of which form the egg-follicles. The egg-strings elongate as the eggs move down into the follicles,

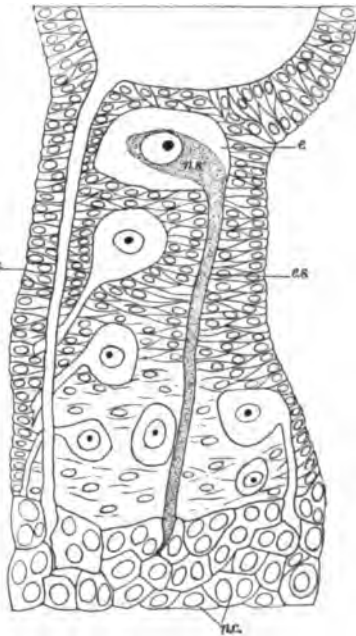


FIG. 1. Longitudinal section of an ovariole showing ovocytes at the beginning of the growth period. The nutritive stream (n.s.) is represented in only one of the eggs (e). e.s., egg string; n.c., nurse cells; o.s., ovariole stalk.

¹ Festschr. Nat. Med. Vereins. Heidelberg.

² Ber. d. Nat. Ges. Freiberg, I.

³ Bolle. Lab. zool. gen. e agr. della R. Scuola Superiore d' Agricoltura di Portici, III., 1908.

and pass back from the proximal end of the egg (which represents the anterior end of the future embryo) to the nurse cells.

When the egg is at the stage of development shown at *e*, the food material passes in a broad stream toward the nucleus beyond which it extends for a short distance. A little later (Fig. 2) the

form of the nutritive stream changes so that now it encloses a central area of newly formed yolk. The nucleus has shifted its position to the distal (posterior) end of the egg, where it appears as a rounded body composed of an acid staining ground substance in which a number of basic staining nucleoli are embedded.

When a portion of the cytoplasm containing the food stream is examined at a high magnification, granules of different sizes are seen distributed on an irregular reticulum (Fig. 3). The granules at their point of entrance into the egg as well as those found in the cytoplasm of the nurse cells are of a uniformly small size; while inside of the egg they are of various sizes, due either to coalescence of the small granules or to an actual growth of individual granules. These granules now pass through an interesting cycle

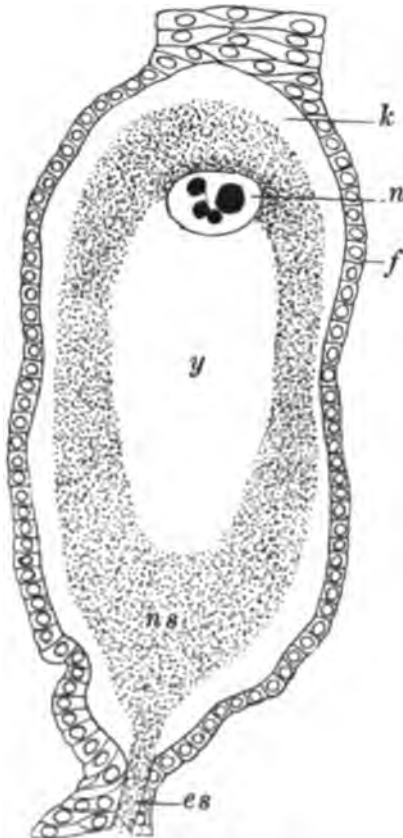


FIG. 2. Longitudinal section of a half mature ovocyte. *es*, egg string; *f*, egg follicle, formed by the cells of the ovarian stalk; *k*, Keimhaut; *n*, nucleus; *ns*, nutritive stream.

of stages. When they have attained their full size they stand out from the reticulum and project into the interreticular spaces (Fig. 4, *a*). They now divide (Fig. 4, *b*). This division is very exact and divides each spherule into two bean-shaped halves.

A series of divisions now ensues until a number of bodies, like chromosomes in appearance, is produced in a single space (Fig. 4, *c*). After about four or five divisions have taken place, they begin to lose their affinity for the basic stain and also their regularity of outline (Fig. 4, *d*), and in the next stage (Fig. 4, *e*) they are seen to have fragmented into numerous irregular bodies that stain with the acid dye. Still later (Fig. 4, *f*) the space is practically filled with a finely granular acid staining yolk mass.

The process of yolk formation, therefore, consists in the transformation of the basic staining granules of the food stream into an acid staining yolk which fills the meshes of the cytoplasmic reticulum of the mature egg. In this transformation the cytoplasm as well as the nucleus is undoubtedly involved.

The yolk is first laid down in the center of the egg, but as the granules spread outward as well as inward from the nutritive stream it is not long before yolk is found on either side of the food stream.

The pole disc does not appear until the egg is nearly mature, and a study of successive stages shows that it is composed of granules of the food stream that have accumulated at the posterior end of the egg (Fig. 5). These granules are much larger than those of the Keimhaut (the peripheral layer of cytoplasm surrounding the yolk in the mature egg) and stain intensely with the basic stain. This staining reaction might suggest that the granules are of nuclear origin; but while the nucleus at this time is far from inactive, with an interchange of materials between it and the cytoplasm probably taking place, I have never been able to observe an actual emigration of granules from the nucleus into the cytoplasm.

Union of the germ nuclei in fertilization and the early cleav-

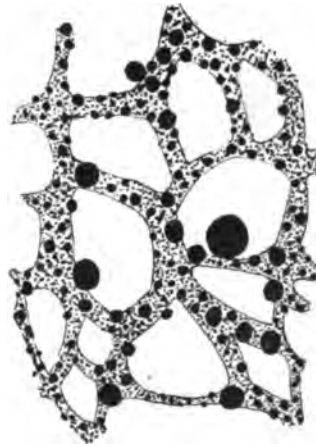


FIG. 3. Section of the cytoplasm of the growing ovocyte in the region of the nutritive stream. $\times 2,000$.

ages take place approximately in the center of the egg. As cleavage proceeds there occurs a separation of the nuclei into two groups, one of which moves outward into the peripheral cytoplasm to form the nuclei of the "Keimhautblastem," while the

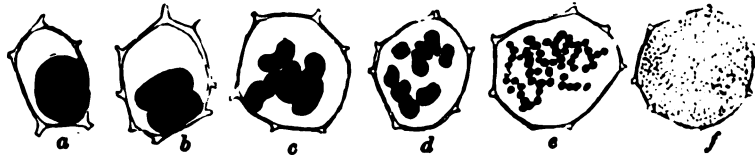


FIG. 4. Six stages in the division of a spherule of the nutritive stream in the process of yolk formation. $\times 1,200$.

other (the vitellophags) remains scattered through the yolk. According to Hegner a definite number of nuclei reaching the posterior part of the egg do not remain in the peripheral cytoplasm, but collect about them a number of granules from the pole disc and continue their migration until they are entirely separated from the blastoderm. These nuclei with their accumulations of cytoplasm constitute the primordial germ cells.

Fig. 6 represents a section taken through the region of the pole disc shortly after the pole cells have reached the periphery. It will be noticed that the granules of the pole disc are much finer than in the earlier stage shown in Fig. 5. The granules in the

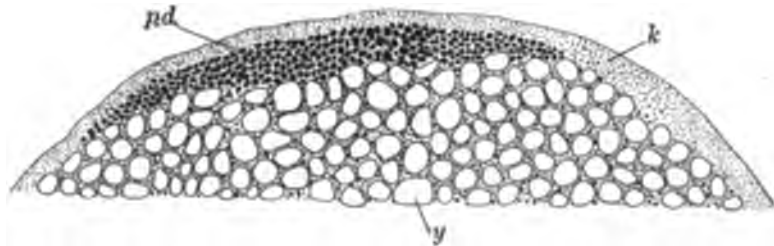


FIG. 5. Longitudinal section of the posterior end of a mature ovum. $\times 1,000$
p.d., pole disc; k., Keimhaut; y., yolk.

cells are distributed on a reticulum and stain intensely with the basic dye, but no more so than the granules that are found in the cytoplasm of the blastema cells. In one case the granules are derived from the pole disc, while in the other they come direct from the food stream. The original source in both cases is the material secreted by the nurse cells.

Does the fact that the granules of the pole disc are derived from the food stream in any way modify our conception of their significance? Were they derived from the chromatin of the nucleus, the fact might be regarded as supporting their hypothetical rôle of germ cell determinants, inasmuch as it would be in accord with the view that the chromatin plays a primary part in directing and perhaps determining the activity of the cell. On

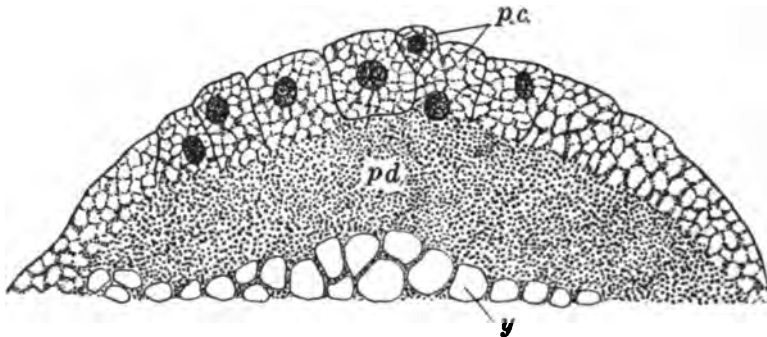


FIG. 6. Longitudinal section of the posterior end of the early embryo. $\times 600$. *p.c.*, pole cells; *p.d.*, pole disc; *y.*, yolk.

the other hand, if they represent so much food material, their significance as germ cell determinants appears in a different light.

The granules of the food stream are derived from the cytoplasmic granules of the nurse cells, which are germ cells that have lost the function of reproduction and that have taken up secondarily the function of secreting material for the growth and development of the ova. In early stages the nurse cells do not differ in any regard from their sister cells destined to become ovocytes. In my paper referred to above I have described what appear to be some of the factors in the differentiation of the primordial germ cells into nurse cells and sex cells, and shall not enter into the matter here.

At the time of this differentiation the nurse cells pass through a period of amitotic divisions, at which time the usual staining reactions of nucleus and cytoplasm become reversed, *i. e.*, the nucleus stains with the acid dye, while the cytoplasm, including the granules which later form the nutritive stream, stains with the basic dye.

The granules may therefore be of the nature of chromatin, and actually represent the chromatin of the nurse cells; for the chromosomes never appear in these cells after they have divided amitotically. But even though this be the case a nuclear origin for these granules loses any special germinal significance when we remember that the latter develop into yolk as well as pole disc, and are therefore just as much "yolk determinants" as "germ cell determinants."

It would appear that a fact of far greater importance in the determining of the germ cells lies in the migration of the pole cells to a position outside of the yolk. This migration isolates these cells and places them in an environment that is entirely unique. It is rather difficult to understand how the absorption of pole disc granules could be the cause of this. The fact that the pole disc occupies a position between the pole cells and the yolk gives a considerable foundation for regarding it as a source of nutrition for these cells.

Hegner states that the granules of the pole disc are absorbed by the pole cells in passing through; after which no further importance is attached to the pole disc. However the granules are not all taken up by the cells in their migration and the greater part of them remains behind after the cells have passed through (Fig. 6).

If then the pole disc represents a part of the nutritive stream of the ovum that has not been transformed into ordinary yolk, but instead has been reserved to supply the pole cells, the conclusion presents itself that the latter as a result of this special kind of nutrition undergo a peculiar method of metabolism which differentiates them from the somatic cells. When these cells are ready to immigrate into the embryo through the pole-cell canal the differentiating factor has already acted and the germ cells are readily distinguished by certain morphological peculiarities from the somatic cells.

It would be highly interesting to know whether or not the germ cells will develop in the absence of the pole disc. The experiments devised by Hegner to test this point have been negative in results, although they do show that the egg may have its contents profoundly disturbed without preventing the production

of a normal embryo. When the granules of the pole disc are displaced by the centrifuge, they are said to move *en masse*, which indicates that they form a structural feature of considerable rigidity.

While more decisive experiments are needed to clear up this point, the evidence at the present time from the morphological side shows that the granules of the pole disc consist of particles derived from the food stream of the ovum that form an accumulation in the protoplasm in its posterior part.

FORTY-NINE GENERATIONS IN THE DARK.¹

FERNANDUS PAYNE.

Since the time Lamarck put forward the well-known theory of the transmission of acquired characters, this subject has been discussed pro and con by many writers; some believing that it is one of the guiding principles in the evolution of species; others that the transmission of an acquired character is an utter impossibility. I shall not attempt a review of the literature, as every book on heredity and evolution has its chapter devoted to that. In fact, it seems to me that there has been too much discussion and not enough experimental work. I believe it can justly be said that there has not been a single decisive experiment which proves or disproves the theory. They are all open to criticism in one way or another. The main argument which the opponents of the theory advance is that there has been no proof brought forward, and further there is no conceivable way in which an acquired character could be transmitted. On the other hand, the supporters believe that this is one of the easiest ways of explaining evolution and as it helps us out of many a difficulty it must be true.

Much interesting data has been collected, but none of it is conclusive. What we need at the present time is more experimental work to test the validity of the theory. With this attitude toward the subject, I started an experiment October 21, 1907, while at Columbia University to test the effect of darkness upon the common fruit fly, *Drosophila ampelophora*, and, if any effect was noticeable, to test its transmissibility. This seemed to be a suitable experiment as the present condition of cave animals is easiest explained by the assumption of the transmission of acquired characters.

The paper is not a finished report, but it may be of interest to scientific men to learn that such an experiment is in progress, and that this fly has been bred in the dark for forty-nine generations. Most certainly the length of time is rather short, but the

¹ Contribution from the Zoological Laboratory of Indiana University. No. 112.

number of generations is large when compared with longer lived animals. Forty-nine generations of mankind would cover about fifteen centuries of time.

The first changes noticeable in cave animals are the loss of color and the degeneration of the eyes. So far, in the case of the flies, no visible change in color has manifested itself. Sections of the eyes showed all parts perfectly normal. Although I have not succeeded in getting an accurate method of measuring it, there is a noticeable difference in their reactions to light. These flies are positively phototactic and if set free nearly always fly toward the light. Those bred in the dark are still positive, but they do not react so quickly nor do so many of them react. At the end of the tenth generation, this difference was noticeable. So noticeable, that when two vials, one containing flies bred in the light and the other in the dark, were shown to several people at the laboratory without them knowing what they were, they immediately remarked that one lot went toward the light much more quickly than the other. This tenth generation, after being bred in the light for one generation, still showed a difference but not quite so marked. Whether or not the flies of the forty-ninth generation are more sluggish in their reactions to light than those of the tenth generation is impossible for me to say as my method of testing is not sufficiently accurate. I expect to continue the experiment and hope later to devise a method by which I can test the reactions of each fly individually and thus see whether the effect of the darkness is a cumulative one.

Much discussion has centered around the question of the origin of cave faunas and several suggestions have been made in regard to its solution, but it seems pretty generally concluded that animals which now inhabit caves have entered them because they were originally dark-seeking forms. The present experiment shows that a positively phototactic animal might establish itself in a cave if it were accidentally enclosed in such a place and if a suitable food supply were present.

Since no changes have been produced in the color of the body or in the structure of the eyes by breeding the fruit fly in the dark for forty-nine generations, the question arises as to whether the length of time which these flies have been bred in the dark

is too short or whether they have reached a fixed condition in so far as variations toward adaptations for a cave life are concerned. We might extend it a little further and ask whether all animals would lose their color or whether their eyes would degenerate in a cave environment? In other words, is the environment alone responsible for the present condition of cave animals or is there an internal orthogenetic factor at work to which the environment serves as a stimulus? Eigenmann in his discussion of the origin of cave animals has stated that forms which now inhabit caves were predestined to become cave animals long before they ever entered a cave. That is, they had varied in a certain fixed direction, and these variations had tended to fit them for a cave existence. If such is the case, would the present cave animals have become blind and colorless if they had not entered caves?

DO BLOW FLY LARVÆ RESPOND TO GRAVITY?

S. O. MAST.

Referring to the fact that blow-fly larvæ crawl under objects in water just as they do outside, Loeb says (1905, p. 68):¹ "In these experiments I was struck by the fact that the animals, when placed under the surface of the water, do not swim upward and so avoid death, but swim downward. I cannot explain this fact. Under other conditions positive geotropism cannot be demonstrated in these animals." Relying on this statement of Loeb, I attempted to ascertain the cause of the expressed difference in response to gravity of fly-larvæ in air and in water and the method of orientation in swimming downward. The result of this investigation follows.

In making observations on the reactions of blow-fly larvæ in air, twenty-five active specimens varying in length from 5 to 17 mm. were put into a glass jar with vertical walls so situated that the light intensity in the jar was very low and approximately uniform throughout, thus eliminating its influence on the direction of movement. In the course of several minutes it was found that nearly all the larger specimens were crawling almost straight upward on the sides of the jar, apparently responding negatively to gravitation. The smaller larvæ however crawled about in all directions, horizontally and downward as well as upward. What is the cause of this difference in the direction of locomotion?

Careful observation soon showed that while most of the larger larvæ observed at any given time were directed upward, they frequently turned and started in other directions, but that as soon as they got into a position approaching the horizontal they either fell to the bottom of the jar or the posterior end swung downward somewhat every time it was raised in the process of looping, and that this resulted in orientation with the anterior end directed upward.

Thus it is clear that there is no evidence of a reaction to gravitation in these forms under the conditions of these observations, *i. e.*, in air. Do they become positive to gravity in water and swim downward as Loeb says, and if so, how do they orient?

Larvæ approximately 6 mm. long were taken from carrion

¹ Studies in General Physiology, Vol. I., 423 pp., Chicago.

and put into a glass jar containing water 20 cm. deep. They all soon reached the bottom, but there was not the slightest evidence of swimming downward. They did not orient and the longitudinal axis was nearly horizontal in many of them. In fact there was but little bending and twisting in the larvæ. Most of them remained perfectly quiet as they sank to the bottom and none of them deflected more than 2 cm. from the vertical. The same was true for larvæ approximately 1 cm. and 1.6 cm. long taken from the carrion and put directly into the water on the second and fifth days following. But in case of other specimens which were kept in a dish without food for several hours before they were put into the water many did not sink. This was due to the formation of small bubbles of gas near the anterior end.

Some of the larvæ which had thus been without food were killed in boiling water, others in alcohol. Of the dead specimens some sank, others floated. The dead ones were now mixed with living ones, and all put into a small glass jar containing water 80 cm. deep. After the larvæ which were heavier than water had sunk to the bottom, the jar with the upper end closed was suddenly inverted. Those which had been at the bottom sank again, while those which had been at the top rose. In both lots there were some living and some dead specimens, but it was only with great difficulty that one could distinguish them. In nearly all specimens the axis was approximately horizontal. There was no evidence whatever of a swimming movement in any of them. If there is no gas in them they sink to the bottom where they crawl about much as they do in air. If they contain bubbles of gas they float at the surface, where in one instance they were observed to remain alive more than twenty-four hours.

It must therefore be concluded that blow-fly larvæ do not react to gravity, either in water or out of it. In air they may be found to orient and crawl nearly straight upward on objects, but this is not due to a response to gravity on the part of the organisms. In water they sink to the bottom or float at the top, depending upon the amount of gas they contain, but there is no evidence whatever indicating that they can swim.

PECULIAR HABITAT OF A PYCNOGONID (ENDEIS
SPINOSUS) NEW TO NORTH AMERICA, WITH
OBSERVATIONS ON THE HEART AND
CIRCULATION.¹

LEON J. COLE.

During the summers of 1904, 1905 and 1906, sargasso, or gulf-weed, at various times drifted into Vineyard Sound in considerable abundance. This is the case some years, while others it may seldom be seen or may be wholly absent during the entire season. Gulf-weed is borne northward each season in large quantities by the Gulf Stream, which here lies nearly a hundred miles off the coast, and its appearance in these in-shore waters would seem to depend in large part upon the occurrence and prevalence of southerly and easterly winds, which divert a certain amount of it shoreward from its course further out. In 1904, and presumably also in the two succeeding seasons, although my records do not state specifically as to this, the weed was covered with a delicate but very abundant growth of the hydroid *Obelia dichotoma* (Linn.).² Living among the colonies of this hydroid, and clinging tenaciously to its stems and branches, occurred a slender pycnogonid, a species of *Endeis*, both adult males (many of them carrying eggs) and adult females, as well as young in various stages of growth, being found in great numbers.

The largest of these pycnogonids were of a considerable size, being approximately 15 mm. across from tip to tip of the extended legs; but all were nearly white, or of a light straw color, in general appearance, which rendered them far from conspicuous among the similarly colored hydroid stems. Examined individually, with more care, it was found that the intestine and its diverticula in the legs usually appeared greenish, probably due to its contents, while there was a pinkish tinge at each of the articulations of the different joints (articles) of the legs. When

¹ The observations reported in this paper were made in the laboratory of the United States Bureau of Fisheries at Woods Hole, Mass.

² The identification of this species was kindly confirmed for me by Professor C. W. Hargitt.

the intestine was empty, the legs often appeared white at the joints (articulations) and transparent between. The distal half of the proboscis is usually the most conspicuous part of the animal, being a tinge or shade of yellow, in some cases so dark as to be almost buff. Eyes dark reddish brown.

While this pycnogonid was found plainly to belong to the genus *Endeis* (*Phoxichilus*¹ of authors), its peculiar occurrence among the gulf-weed, and the fact that the genus had never been reported from American waters, led to the suspicion that it would prove to be specifically distinct from any described form. Comparison, however, with descriptions of *Endeis spinosus* (Montagu) of the European coast showed so little discrepancy that I have delayed coming to a definite conclusion until I should be able to compare it directly with specimens from that locality. This I have now been enabled to do through the kindness of Prof. G. O. Sars, of Christiania, Norway, and Mr. T. V. Hodgson, of Plymouth, England, who have generously supplied me with

¹ The name for this genus has been commonly known and accepted as *Phoxichilus* since 1837 at least. It now appears that the first form described as a *Phoxichilus* in reality belongs to Wilson's genus *Pseudopallene*. According to the iron-clad "law of priority" therefore, the name *Phoxichilus* must now be used in place of *Pseudopallene* for that genus, and hereafter all but specialists on Pycnogonida will find what is to them an almost hopeless confusion of names if they ever have occasion to look up the literature on such a subject, say, as the circulation of the blood in this group, mentioned later in this paper. Stebbing (1902, p. 187) proposed the name *Chilophoxus* to take the place of *Phoxichilus* for those animals which have been universally known by the latter name. But the evil ball once started rolling could not be stopped here, for Norman (1908, p. 231) has pointed out that undoubtedly the first species described by Philippi (1843) under his genus *Endeis* "is congeneric at least, if not identical, with *P[halangium] spinosum* Montagu," which has so long been known as *Phoxichilus spinosus*, and is the subject of the present note. This being the case (and there would appear to be no doubt of the facts) *Endeis* of course takes precedence over *Phoxichilus*, and the name must therefore stand as *Endeis spinosus* (Montagu).

When I began systematic work on the Pycnogonida, more than ten years ago, I believed implicitly in the advantages to be gained by following strictly the law of priority; after considerable experience in observing its effects, I am inclined to agree with Thompson (1909, p. 537, footnote) when he remarks with regard to the instance just discussed: "In my opinion this is a case where strict adherence to priority would serve no good end, but would only lead to great and lasting confusion." However, after much mental contention, I have decided for the present to be law-abiding, and to live in the hope that before long an agreement may be reached among zoölogists permitting the use of common sense in place of forcing blind adherence to a law which, however good its intention, has seemed only to increase the instability and confusion in zoölogical nomenclature.

specimens of undoubted *Endeis spinosus* from the Norwegian coast and from Plymouth, respectively. Furthermore, I have had one specimen, labelled "*Phoxichilus vulgaris*," which I feel confident came from Naples, although no locality was given on the label. My reasons for believing that this specimen came from there are twofold: First, because it was with other Mediterranean pycnogonids, and second, because the name *vulgaris*, which European writers unanimously consider as a synonym of *spinosus*, has been used for this species only in that locality. Finally, in addition to those from Vineyard Sound I have from the American side of the Atlantic a number of specimens of *Endeis* from the Tortugas, Florida. One of these I collected myself; the others were kindly sent to me by Dr. A. G. Mayer, director of the Marine Laboratory of the Carnegie Institution.

After having compared carefully the specimens from these five widely separated localities, viz., Naples, Plymouth, Norway, Vineyard Sound and the Tortugas, I am forced to the conclusion that in spite of their range and their difference in habitat, they in reality must all be considered as belonging to a single species, *Endeis spinosus* (Montagu). The Vineyard Sound specimens, it is true, appear in general a little stouter than those from Norway, due to the legs being proportionately a trifle shorter; this difference, however, is not great and is inconstant. The following table presents a number of proportions, based usually on the average of measurements of all the legs of from one to three or four specimens from each locality. The numbers of specimens measured were so small and the results are in general so irregular that not much importance can be attached to them. It will be noticed that in the proportion of the length of the femur to that of the coxal region, there is a gradual relative shortening of the femur as one goes from Naples up the European coast and down the American side to the Tortugas; the same is true in a general way for the relation of the trunk to the proboscis; while the relation of the trunk to total leg length is more irregular. This gradation in two of the cases may or may not have any real significance.¹

¹ Owing to the comparatively small amount of differentiation in the four pairs of walking legs of the pycnogonids and to the fact that they present characters

Proportionate Measurements of Endeis spinosus from Different Localities.

Localities.	Ratios.		
	Coxa to Femur.	Leg to Trunk.	Proboscis to Trunk.
Naples	1 : 1.80	1 : 3.55	1 : 2.35
England	1 : 1.70	1 : 4.14	1 : 2.01
Norway	1 : 1.68	1 : 3.85	1 : 2.14
Vineyard Sound, ad.	1 : 1.66	1 : 4.15	1 : 1.83
" " juv.	1 : 1.32	1 : 3.26	1 : 2.03
Tortugas, ad.	1 : 1.43	1 : 3.74	1 : 1.92
" " juv.	1 : 1.29	1 : 3.53	1 : 1.89

Of even more interest than the mere fact of the occurrence of *Endeis spinosus* on the American coast is its peculiar pelagic habitat on this side of the Atlantic. So far as I am aware, a pelagic habit among the Pycnogonida has not heretofore been reported, either for this species, which in Europe has been recorded as dredged from shallow depths, or any other. In a previous paper (Cole, 1901, p. 197) I have called attention to the fact that *Pallene brevirostris*, a very slender pycnogonid which occurs at Woods Hole, swims actively if by chance it becomes free in the water; but ordinarily it lives among the hydroids and algæ growing attached in shallow water. Curiously enough the specimens of *Endeis* from the Tortugas sent me by Dr. Mayer are all recorded as having been obtained from the "surface" or in the "tow." And in addition to these he has also sent me several specimens of *Pallene* and *Nymphon* (species as yet undetermined) which were obtained in the same manner. While enjoying the privileges of the Carnegie Laboratory at

(linear dimensions) which can be measured with ease and accuracy, it would seem that these animals present exceptionally favorable material for a statistical study of differentiation and correlation of these appendages. What observations and measurements I have made would seem to indicate, for example, that the ratio of the length of the legs to the body, and the correlation between the legs themselves, varies with the age of the animal, and that there is a positive correlation between the age of the individual and the position of the legs from behind forward. In other words, the posterior pairs of legs present more juvenile characters than the anterior ones. Such a study could be made profitably only on a species which could be obtained in abundance at all stages of growth. The species under consideration would be excellent for such a study, and *Anoplodactylus lentus* is another that may be obtained abundantly at Woods Hole. Whatever else of general interest might be learned, the results would be of the greatest value to the systematist in this group, who has usually to deal with a small number of specimens taken at scattered localities.

the Tortugas in 1906, I myself obtained a live specimen of *Endeis spinosus*, the animal appearing in a finger bowl which I had just filled with sea water by means of a hand pump used for that purpose, thus showing that the pycnogonid must have been swimming freely in the water when it was sucked in by the pump. Whether these pycnogonids which were taken at the surface had merely become accidentally detached from their regular abodes, or whether certain species naturally swim freely in the water at certain times, I am not prepared to say.

In the present connection the reaction of certain pycnogonids to light is of considerable interest. I have shown (Cole, 1901) that *Anoplodactylus lentus* and *Pallene brevirostris*, both of which can sustain themselves in the water by swimming,¹ move uniformly toward a source of light of moderate intensity (diffuse daylight). I was, furthermore, able to prove the same thing for *Endeis*, at least for the one specimen mentioned above as secured by myself at the Tortugas, the reaction being in all respects similar to that previously described for the other genera. The biological significance of this reaction in the case of *Endeis*, living among the hydroids on the gulf-weed, is at once apparent, for any individual which by chance became detached from the hydroid would, in response to the light coming from above, swim upward instead of going down into the deeper water, and would in this way stand a much better chance of again finding suitable attachment. From the fact that the young of all stages appeared to be clinging as tenaciously to the hydroids as the adults, I am inclined to believe that they probably do not have a definite free swimming stage, but swim only when circumstances make it necessary.

The newly hatched larvæ possess strongly developed chelifores and stout chelæ by which they attach themselves to any available object. Normally they remain clinging to the egg cluster on the legs of the male for some time after hatching. The usefulness of these effective grasping organs is readily apparent, since without them the larvæ would almost inevitably become detached and lost from their floating abode. I have elsewhere (Cole, 1904, p. 316) pointed out the usefulness of the chelifores

¹ The "swimming" consists simply of a vigorous *kicking*.

in preventing pycnogonid larvæ from being swept off the hydroids by a swift tide. After metamorphosis the claws of the walking legs are used for attachment, while the chelifores then probably serve as feeding organs, as has been observed to be the case in *Anoplodactylus* (Cole, 1906) and *Phoxichilidium* (Loman, 1907).

It seems worth while to call the attention of any who might care to investigate the embryology or anatomy of this group to the advantages of this species should it continue to appear in Vineyard Sound, where it is so readily accessible to the marine laboratories at Woods Hole. During the seasons it was observed all stages for a study of the embryology and later development were present in great abundance, while owing to its transparency, it is very favorable for observing the internal anatomy, as well as some of the physiological functions of the living animal. The peristaltic movements of the intestinal ceca in the legs are easily discernible, and our knowledge of the circulation of the blood in the pycnogonids has been derived largely from this species. The passage of food through the intestine and the extrusion of feces has been well described by Loman (1907) in the case of *Phoxichilidium femoratum*.¹ The question of the circulation has been touched upon by a number of observers, being most thoroughly considered by Dohrn (1881), but as this author makes no mention of earlier writers, it may be well to give here a brief résumé of the literature on the subject, together with a few additional observations.

Johnston (1837, pp. 374 and 379) appears to have been the first to mention a circulation of the fluids in the pycnogonid, his observations being made on the living "*Orithyia coccinea*" (= *Phoxichilidium femoratum*). Johnston, however, apparently mistook the branching intestine for blood vessels, for he says (p. 374): "The circulating system is probably reduced to a single vessel which occupies the centre of the thoracic segments, and sends a branch to each member or limb, in which the blood has an irregular movement, but cannot be said properly to circulate." On p. 379 he adds that "there is no heart." Milne-Edwards, in his "*Histoire naturelle des Crustacés*" (1840, p. 531), dismisses

¹ *Phoxichilidium femoratum* is well known on our coast to the northward of Cape Cod.

the matter with the statement that there exists "une circulation vague." According to de Quatrefages (1845, pp. 75, 76) no heart or vascular system exists, but the blood is agitated back and forth in part by the movements of the legs and also by the muscular movements of the intestine, which he believed to lie freely in the internal space of the legs. Van Beneden (1846, pp. 72, 73), observing a living *Nymphon*, was the first to make out a regular circulation. He states that the blood may be seen to flow down one side of a leg and back the other, then into the next following leg, and so on to the last pair, after which he could not tell what course it takes. On account of the opacity of the intestine he could not determine whether there was a heart or dorsal vessel present, but he observed a contractile membrane at the bases of the legs. (It is probable that what he saw were the valves of the heart where that organ extended beyond the outline of the intestine.)

Zenker (1852, pp. 382, 383), also studying *Nymphon*, was apparently the first really to see the heart. He describes it as a thin-walled sac with ramifying muscle fibers, the contour being most clearly discernible in the region of the last pair of legs. Three years later Krohn (1855) published a much fuller description of the circulation, together with a figure of the heart of *Endeis spinosus*. Hoek (1881a, 1881b) described the structure of the heart in *Colossendeis* and certain other forms. According to him there are ordinarily three pairs of lateral ostia (the posterior pair being very close together) except in *Pallene brevirostris*, which has but two pairs. In the same year Dohrn (1881) published the best description of the structure of the heart and of the circulation that has yet been given, his description being based principally again on *Endeis*. He found here two pairs of lateral ostia, with commonly, but not always, an unpaired aperture at the posterior end. There are no blood vessels aside from the heart, but the blood driven out from the anterior end of the heart is forced into the proboscis and runs back along the ventral side of the body, and from here it flows out into the legs. In general the direction of flow is centrifugal on the ventral side and centripetal on the dorsal side (except in the proboscis, where it is just the reverse). A thin membrane, which supports the

intestine, keeps the two streams apart (cf. Fig. 2, *sept.*). Dohrn's description of the heart agrees very closely with that of Hoek, it being an elongated sac with thin muscular walls, which, how-

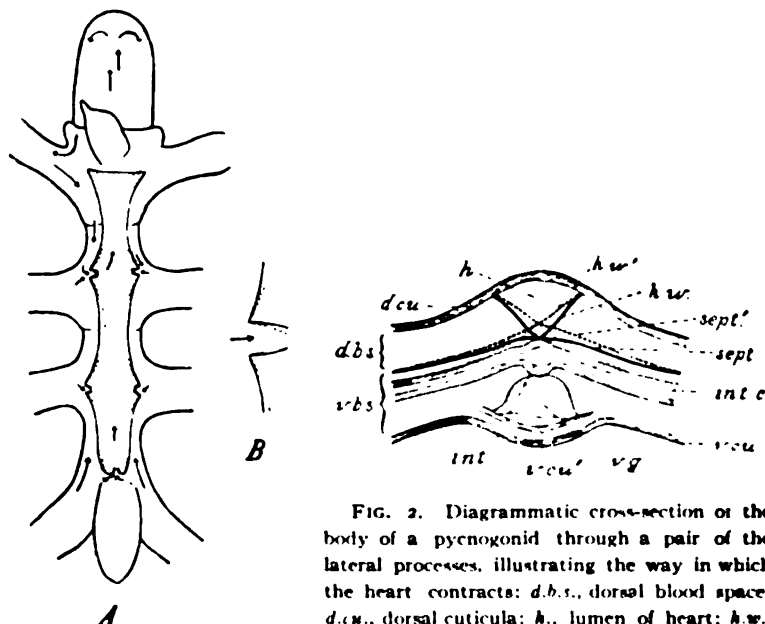


FIG. 1. A, apparent change in the shape of the heart with contraction and expansion, as seen when viewed from above in a living specimen of *Enderis spinosus*. Solid line, diastole; broken line, systole. The arrows show the course of the blood where its streaming could be observed; the opacity of the intestine and other organs prevented its being seen in other parts. B, a single lateral ostium with valve.

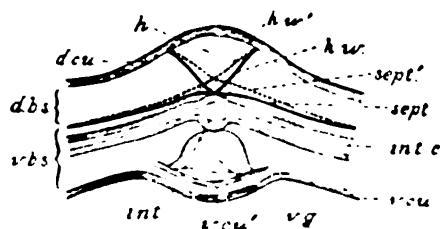


FIG. 2. Diagrammatic cross-section of the body of a pycnogonid through a pair of the lateral processes, illustrating the way in which the heart contracts: d.b.s., dorsal blood space; d.cu., dorsal cuticula; h., lumen of heart; h.w., side wall of heart (at diastole); h.w.', position of side wall of heart at systole; int., main longitudinal trunk of intestine; int.c., intestinal caecum going to leg; sept., transverse horizontal septum (at diastole); sept.', position of transverse septum at systole; v.b.s., ventral blood space; v.cu., cuticula of ventral side, v.cu.', posterior edge of underlapping cuticula of the preceding segment; v.g., ventral ganglion. (Based on the cross-section of a species of *Nymphon* figured by Dohrn, 1881, pl. 15, fig. 10. The ovary, which lies between the intestinal caeca and the transverse septum, has been omitted for the sake of clearness.)

ever, do not completely enclose it, the dorsal wall being formed by the chitinous integument of the back (cf. Fig. 2).

In *Phoxichilidium femoratum* Loman (1907) found that a systole of the heart occurred two or three times each second, which would be 120 to 180 contractions to the minute. A count

of the rate of the heart beat of *Endeis spinosus* which I made at Woods Hole, September 4, 1904, showed 172 contractions per minute, the rate slowing down in a short time after the animal had been mounted under a cover glass. The next morning other specimens showed a rate of 126 to 136 beats per minute. Fig. 1, from a rough sketch made at the time, shows the position and shape of the heart; the dotted lines indicate the apparent change in shape as the muscular side walls contract, and the arrows show the direction of observable streaming of the corpuscles in the body fluid or blood. It must be remembered that owing to the attachment of the side walls to the dorsal integument they must be stationary at this point, the contraction of the muscles in the side walls tending to draw the sides together further down, thus reducing the capacity of the enclosed space (see Fig. 2). It is probable that with the alternate contraction and relaxation of the heart the transverse septum (*sept.*), which divides the body space into dorsal and ventral chambers, is raised (Fig. 2, *sept.*) and lowered to some extent, which would help to force the blood out into the legs at each systole and to draw blood from the legs into the pericardial space at the diastole.

The two pairs of lateral ostia, opposite the lateral processes of the body for the attachment of the second and third pairs of legs respectively, could be plainly distinguished; the single opening at the posterior end was somewhat less distinct. The streaming of the blood cephalad through this portion of the heart left no doubt, however, of the existence of such an opening, except in one case in which the blood could be seen to move back and forth in this region without a definite streaming forward. This would seem to confirm Dohrn's observation that in some cases there is no posterior terminal ostium, or at least it appears that if it existed in this instance it was closed and not functioning.

Although, on the whole, there appeared to be a real circulation from the body out into the legs and back, this was rendered more or less indefinite by the peristaltic contractions of the intestine, which imparted a sort of churning motion to the blood and kept it moving back and forth. This was especially evident in the more expanded femoral joints; in the basal parts of the leg and in the tibia a more definite streaming could be observed. The

contractions of the intestinal ceca in the legs are more or less irregular. There were usually a few (two to four) peristaltic waves in one direction, and then they would change and go the opposite way for an equal time. In some cases the contraction appeared to start at one point and pass from there in both directions along the intestine. In a specimen which had been mounted for some time under a cover glass these peristalses recurred at intervals of two or three seconds.

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THE STRUCTURE OF THE SPERMATOPHORES OF AMBYSTOMA PUNCTATUM.

BERTRAM G. SMITH.

Since the recent paper of Wright and Allen ('09) has again directed attention to the breeding habits of *Ambystoma punctatum*, it has occurred to me that a more careful description of the spermatophores than has yet been given might be of value in a comparative survey of the habits of the urodeles. No adequate figures of these spermatophores have yet been published.

The spermatophores of *Ambystoma punctatum* studied by me at Ann Arbor (Smith, '07) were not freshly deposited, and were not in the best possible structural condition for study; hence only a brief description, illustrated by a photograph, was attempted. Since then I have been able to study these structures in abundance under the best possible conditions. As evidence that the spermatophores were freshly deposited, there may be cited the fact that the spermatozoa of some of them were found to be active when examined in the laboratory seven hours after their collection. The results of this study have confirmed the description previously given by me, in regard to distribution and general structure, but have revealed the common occurrence of compound spermatophores, a condition previously unnoticed.

In the early spring of 1909, when the snow had nearly all disappeared, I found specimens of *Ambystoma punctatum* and *A. jeffersonianum*, under rocks on the hillside near "Branchipus Pond," about two miles from the campus of Syracuse University. The study of the habits of these animals was undertaken in order, if possible, to determine by direct observation the exact procedure in the process of fertilization by means of spermatophores in the case of *A. punctatum*, and to obtain data in regard to *A. jeffersonianum*, in which the manner of fertilization is entirely a matter of conjecture.

Conditions made it impossible to keep a very close watch of the pond during the breeding season, and the abruptness of the weather changes caused me to miss the few opportunities for direct

observation of the activities of the animals in the field, by means of a lantern at night. Specimens of both sexes were transported to the laboratory and kept under observation in aquaria; several males of *A. punctatum*, and one of *A. jeffersonianum*, deposited masses of seminal fluid on the floor of the aquarium, but nothing resembling the formation of true spermatophores occurred. The largest of these masses was nearly 2 cm. long, and the quantity of sperm deposited by each individual was amply sufficient to form a group of spermatophores. The behavior of the sexes toward each other presented nothing that could be interpreted as normal breeding behavior.

In the pond large numbers of spermatophores were deposited, and these were carefully studied, both *in situ* and in the laboratory; but all the specimens collected were identified, with considerable certainty, as the product of *A. punctatum*. The failure to solve the main problems caused me to lay my notes and drawings aside, awaiting an opportunity to continue the investigation; but the conclusive results of Wright and Allen ('09) have made this unnecessary so far as regards *A. punctatum*.

Identification of Species.—The fact that *A. jeffersonianum* occurs, though less abundantly, in the same region with *A. punctatum* made necessary a determination of the species of the spermatophores found. The only available method seemed to be through a study of the spermatozoa. The material available consisted of spermatozoa from a considerable number of individuals of *A. punctatum*, taken both at Syracuse and at Ann Arbor, and spermatozoa from one specimen of *A. jeffersonianum*, taken at Syracuse. Material from each individual was fixed by a variety of methods, stained, mounted and carefully measured under high powers of the microscope. The average measurements were as follows:

	Acrosome	Head.	Middle Piece.	Tail.	Total Length.
<i>A. punctatum</i>	20 μ	116 μ	15.0 μ	480 μ	631.0 μ
<i>A. jeffersonianum</i>	17 μ	114 μ	11.3 μ	348 μ	490.3 μ

It will be seen that the sperm of *A. jeffersonianum* is in general shorter than that of *A. punctatum*. The middle piece is most easily and accurately measured, and affords the readiest means

of differentiating the two species. The range of variation in the length of the middle piece of *A. punctatum* (in several individuals) is $14\ \mu$ – $16\ \mu$; in *A. jeffersonianum* (in one individual) $11.2\ \mu$ – $11.9\ \mu$.

The fact that only one individual of *A. jeffersonianum* was considered of course militates against the acceptance of these results as absolutely conclusive; but since material was taken from several individuals of *A. punctatum*, and in all of these there was a decided difference from the results obtained from the single individual of *A. jeffersonianum*, it is highly probable that the species may be distinguished in this way.

Spermatozoa were then taken from a dozen or more spermatophores, each from a different group and presumably deposited by a different individual. This material was treated precisely as in the case of the spermatozoa described above. Somewhat to my surprise and disappointment, all the specimens conformed to the dimensions of the sperm of *A. punctatum*.

Structure of the Spermatophores.—Two general types of spermatophores are recognizable: simple and compound. The compound spermatophores may be roughly divided into the vertically serial, and the Y-shaped types, the former by far the most common; but both these conditions may be found combined in one compound spermatophore.

A spermatophore of the simple type is illustrated in Fig. 1. It consists of an expanded hummocky base and a stout stalk, of very clear, transparent, gelatinous material, surmounted by a dome-shaped mass of snowy-white seminal fluid.

While to the naked eye the stalk of a simple spermatophore is perfectly clear, under a low power of the microscope it is seen to possess a delicate network of fibrous material. Under high power this is found to be made up of sparsely distributed spermatozoa, interlaced to form a meshwork. In some spermatophores the strands of this network assume considerable regularity. There may be distinguished regular parallel strands extending in the direction of the axis of the stalk, distributed at about equal intervals through its substance; these strands are connected by more irregular cross-strands.

Evidently a few spermatozoa are present in the cloaca at the

time when the stalk of the spermatophore is laid down, and these spermatozoa owe the regularity of their distribution to the papillated ridges of the cloaca.

For comparison a drawing (Fig. 2) of a spermatophore of *Diemyctylus viridescens*, obtained from a specimen in captivity, is placed alongside the figure of the simple spermatophore of

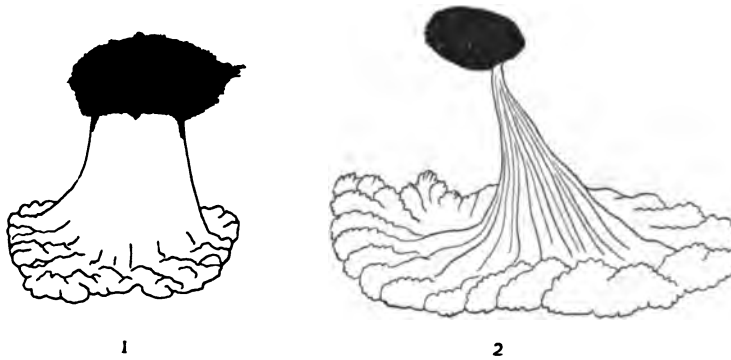


FIG. 1. Camera drawing of simple spermatophore of *Ambystoma punctatum*, $\times 5$. In this and in the following figures the blackened portion is in nature snowy white.

FIG. 2. Camera drawing of a spermatophore of *Diemyctylus viridescens*, $\times 5$.

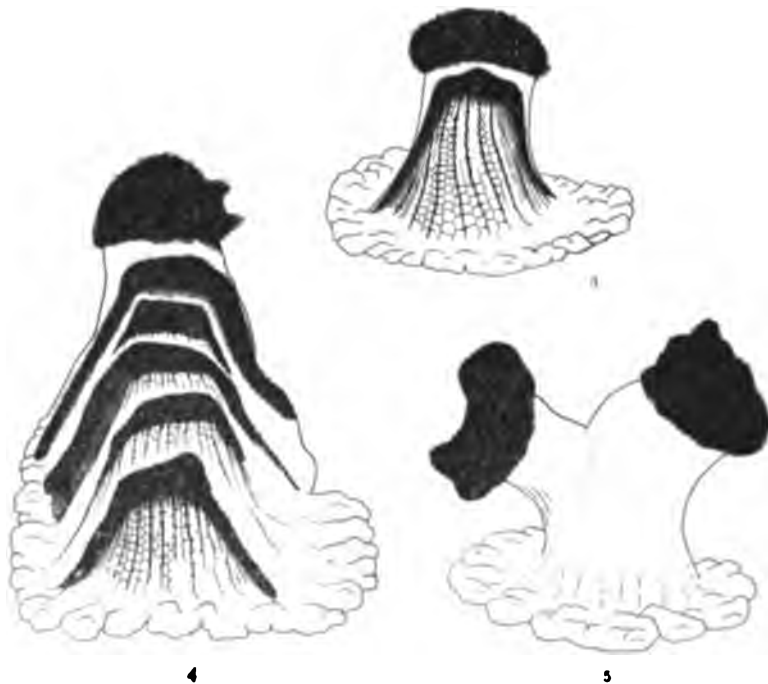
Ambystoma punctatum. (The spermatophore of *Diemyctylus* has been previously described and figured by Jordan, '93.) Several spermatophores of *Diemyctylus viridescens* were available for this study, but none of these differed essentially from the one figured. The average dimensions of twelve simple spermatophores of *Ambystoma punctatum* taken at random, and of two uninjured spermatophores of *Diemyctylus viridescens*, are as follows:

	Total Height.	Longest Diameter of Sperm Mass.	Smallest Diameter of Stalk.	Breadth.
<i>A. punctatum</i>	6.2 mm.	3.7 mm.	2.5 mm.	6.4 mm.
<i>D. viridescens</i>	8.0 "	2.5 "	0.3 "	12.0 "

The simplest and most common case of compound spermatophore is that in which one spermatophore is deposited directly on top of another (see Fig. 3). In the process of deposition of the second spermatophore the sperm ball of the first one is crushed into the form of an inverted cup covering the stalk of the first, and the sides of this cup are stamped with a characteristic

pattern by the fluted surface of the cloaca. This pattern reminds one of the fainter meshwork seen with the aid of a microscope in the stalk of a simple spermatophore.

By an increase in the number of spermatophores arranged in a vertical series we obtain the extreme form of the serial type shown in Fig. 4, which is made up of six simple spermatophores.



FIGS. 3, 4 and 5. Compound spermatophores of *Ambystoma punctatum*. All the figures are camera drawings. - 5.

FIG. 3. Compound spermatophore consisting of two simple spermatophores, one superimposed on the other.

FIG. 4. Compound spermatophore of the vertical-serial type.

FIG. 5. Simplest case of the Y-shaped type.

Somewhat rarely, compound spermatophores of the Y-shaped type (Fig. 5) are found. These are apparently formed by the deposition of one spermatophore on the side of the stalk of another. In several cases noted, one arm of the Y-shaped stalk was again compound, consisting of two or more spermatophores in series.

Compound spermatophores are not all so regular in form as those figured. Sometimes successive spermatophores are packed together so closely that it is difficult to distinguish individuals in the complex; but while the first impression obtained from some of these spermatophores was that of great irregularity, closer study showed that all of them might be reduced to the vertical-serial type, the Y-shaped type, or a combination of these.

Some of the sperm masses of the simple spermatophores, or the terminal one in the case of a compound spermatophore, were found considerably frayed as if from being taken up into the cloaca of a female; rarely, the entire ball of sperm was missing. It is of course possible that either condition might be accidental.

In one hundred spermatophores taken at random, the number of each kind was as follows:

1. Simple type.....	23
2. Compound, two in vertical series.....	45
3. Compound, more than two in vertical series.....	21
4. Y-shaped, or combination of this type with (2) or (3).....	11
Total.....	100

In none of the spermatophores examined was there any approach to the complexity of structure shown in European urodeles by Zeller ('05). Zeller's drawings appear to me highly conventionalized.

Discussion.—Of the various kinds of spermatophores of *Ambystoma punctatum*, the simple type is functionally the most perfect; yet even this is not so highly differentiated and structurally so well adapted for its purpose as the spermatophore of *Diemyctylus viridescens*. In case the of *Diemyctylus*, I have seen almost the entire ball of sperm removed from the very slender stalk by the female cloaca. In *Ambystoma*, on account of the thickness of the stalk affording a large surface for contact with the mass of spermatozoa, this would be more difficult, and according to the observations of Wright and Allen ('09) does not occur—only a very few sperms, comparatively, are detached and retained by the cloaca.

The only mention of compound spermatophores that I find in the literature is in the case of the axolotl, described by Gasco ('81). While in many cases the spermatophores deposited were

single, in one case the male was observed to return repeatedly, at intervals of several minutes, to the same spermatophore and deposit another on top of it, thus building up a compound spermatophore consisting of seven simple spermatophores. Whether the compound spermatophore of *Ambystoma punctatum* is built up in this way I have no means of deciding; it would seem quite as probable that the spermatophores were deposited in rapid succession, while the animal remained in one place—a condition not far removed from what occurs in external fertilization, in which a large amount of seminal fluid is discharged at once. In any case it is difficult to see that any advantage is gained by depositing one spermatophore on top of another and thereby destroying the efficiency of the one previously deposited. As already noted in the case of *Ambystoma punctatum*, the spermatozoa of these spermatophores retain their vitality in water for many hours. The formation of compound spermatophores of the vertical-serial type seems to me a useless and wasteful procedure, and the large proportion of such spermatophores indicates that the spermatophore-forming habit is not highly evolved nor perfectly adapted to its purpose.

In my previous paper (Smith, '07) on the breeding habits of *Ambystoma punctatum*, I ventured the prediction, based on the large number of spermatophores and the manner of their distribution, that the behavior of the adults in the fertilization process would be found to be simpler than in the case of *Diemyctylus viridescens*. In the latter case only a very few spermatophores are formed by each male, and particular safeguards are necessary in order to insure the delivery of at least one of them to the female cloaca; these safeguards are furnished by the complicated but definite series of acts on the part of the adults which precedes and conditions the deposition of a spermatophore. Just as in the higher vertebrates economy of egg production is correlated with care of the young, so in *Diemyctylus* economy in the production of spermatophores is accompanied by a considerable amount of certainty as to their fate. This prediction as to the relative simplicity of the behavior of the adults of *Ambystoma punctatum*, the recent observations of Wright and Allen ('09) have abundantly justified. In the light of the observations on

the structure of the spermatophores recorded in the present paper, we can go further and say that the entire process of securing fertilization by means of spermatophores is in *Ambystoma punctatum* more primitive, less highly adapted to secure its object with economy of material, than in *Diemyctylus viridescens*.

ZOOLOGICAL LABORATORY,
UNIVERSITY OF WISCONSIN,
MADISON, WIS.

LITERATURE.

Gasco, F.

'81 Les Amours des Axolotls. Zool. Anz.. 4.

Jordan, Edwin O.

'93 The Habits and Development of the Newt (*Diemyctylus viridescens*).
Journ. Morphol., Vol. VIII., No. 2.

Smith, Bertram G.

'07 The Breeding Habits of *Ambystoma punctatum* Linn. Am. Nat., Vol. XLI.,
No. 486. (Includes a more extensive bibliography of earlier literature.)

van Leeuwen, W. Docters

'07 Ueber die Aufnahme der Spermatophoren bei *Salamandra maculosa* Laur.
Zool. Anz., 31, pp. 649-653.

Wright, Albert Hazen

'08 Notes on the Breeding Habits of *Ambystoma punctatum*. Biol. Bull., Vol.
XIV., No. 4.

Wright, Albert H., and Allen, Arthur A.

'09 The Early Breeding Habits of *Ambystoma punctatum*. Am. Nat., Vol.
XLIII., No. 513.

Zeller, E. v.

'05 Untersuchungen über die Samenträger und die Kloakenwulst der Tritonen.
Zeitschr. wiss. Zool., Bd. 79.

BIOLOGICAL BULLETIN

I. TRUSTEES

AUGUST, 1909

EX OFFICIO

- F. R. LILLIE, *Director*, University of Chicago.
GILMAN A. DREW, *Assistant Director*, University of Maine.
D. BLAKELY HOAR, *Treasurer*, 161 Devonshire Street, Boston, Mass.
THOS. H. MONTGOMERY, JR., *Clerk of the Corporation*, University of Pennsylvania.

TO SERVE UNTIL 1913

- NATHANIEL L. BRITTON..2965 Decatur Avenue, Bronx, New York City.
S. F. CLARKEWilliams College, Williamstown, Mass.
CHARLES COOLIDGE.....Ames Building, Boston, Mass.
C. R. CRANE.....2559 Michigan Boulevard, Chicago, Ill.,
President of the Board.
ALFRED G. MAYER.....Carnegie Institution, Washington, D. C.
T. H. MORGAN.....Columbia University.
ERWIN F. SMITH.....United States Department of Agriculture,
Washington, D. C.
E. B. WILSON.....Columbia University.

TO SERVE UNTIL 1912

- M. J. GREENMAN.....Wistar Institute of Anatomy and Biology,
Philadelphia.
C. W. HARGITT.....Syracuse University.
C. A. HERTER.....University and Bellevue Medical School,
New York City.
H. S. JENNINGS.....Johns Hopkins University.
GEORGE LEFEVRE.....University of Missouri.
WILLIAM LIBBEY.....Princeton University.
A. P. MATHEWS.....University of Chicago.
G. H. PARKER.....Harvard University.

TO SERVE UNTIL 1911

- H. C. BUMPUS.....American Museum of Natural History,
New York City.
W. A. LOCY.....Northwestern University, Evanston, Ill.
JACQUES LOEB.....University of California, Berkeley, Calif.
F. P. MALL.....Johns Hopkins University.
GEORGE T. MOORE.....Washington University, St. Louis.
L. L. NUNN.....Telluride, Colo.
JOHN C. PHILLIPS.....299 Berkeley Street, Boston, Mass.
C. O. WHITMAN.....University of Chicago.

TO SERVE UNTIL 1910

- E. G. CONKLIN.....Princeton University.
ROSS G. HARRISON.....Yale University.
CAMILLUS G. KIDDER....27 William Street, New York City.
M. M. METCALF.....Oberlin College, Oberlin, Ohio.
WILLIAM PATTEN.....Dartmouth College, Hanover, New Hampshire.
D. P. PENHALLOW.....McGill University, Montreal, Canada.
JACOB REIGHARD.....University of Michigan, Ann Arbor, Mich.
W. B. SCOTT.....Princeton University.

II. ACT OF INCORPORATION

No. 3170.

COMMONWEALTH OF MASSACHUSETTS

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord ONE THOUSAND, EIGHT HUNDRED AND EIGHTY-EIGHT.

HENRY B. PIERCE,
Secretary of the Commonwealth.

[SEAL.]

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The annual meeting of the members shall be held on the second Tuesday in August, at the Laboratory, in Woods Hole, Mass., at 12 o'clock noon, in each year, and at such meeting the members shall choose by ballot a Treasurer and a Clerk, who shall be, *ex officio*, members of the Board of Trustees, and Trustees as hereinafter provided. At the annual meeting to be held in 1897, not more than twenty-four Trustees shall be chosen, who shall be divided into four classes, to serve one, two, three, and four years, respectively, and thereafter not more than eight Trustees shall be chosen annually for the term of four years. These officers shall hold their respective offices until others are chosen and qualified in their stead. The Director and Assistant Director, who shall be chosen by the Trustees, shall also be Trustees, *ex officio*.

II. Special meetings of the members may be called by the Trustees, to be held in Boston or in Woods Hole at such time and place as may be designated.

III. The Clerk shall give notice of meetings of the members by publication in some daily newspaper published in Boston at least fifteen days before such meeting, and in case of a special meeting the notice shall state the purpose for which it is called.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. The Trustees shall have the control and management of the affairs of the Corporation; they shall present a report of its condition at every annual meeting; they shall elect one of their number President and may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

VI. Meetings of the Trustees shall be called by the President, or by any two Trustees, and the Secretary shall give notice thereof by

written or printed notice sent to each Trustee by mail, postpaid. Seven Trustees shall constitute a quorum for the transaction of business. The Board of Trustees shall have power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient.

VII. The President shall annually appoint two Trustees, who shall constitute a committee on finance, to examine from time to time the books and accounts of the Treasurer, and to audit his accounts at the close of the year. No investments of the funds of the Corporation shall be made by the Treasurer except approved by the finance committee in writing.

VIII. The consent of every Trustee shall be necessary to a dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be given to the Boston Society of Natural History, or some similar public institution, on such terms as may then be agreed upon.

IX. These By-Laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-Laws will be acted upon.

X. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

IV. REPORT OF THE TRUSTEES

In making their last annual report the trustees had to announce, with deep regret, the retirement of Professor Whitman from the directorship of the laboratory. As was stated in that report, Professor Whitman was succeeded, in August, 1909, by Professor Frank R. Lillie, whose annual report is submitted herewith. At the same time Professor Gilman A. Drew was appointed to succeed Professor Lillie as Assistant Director. The trustees desire to express their satisfaction that the high ideals of work and the spirit of coöperative endeavor, which the laboratory has so long striven to uphold, have been fully maintained, and that the institution has every reason to look hopefully to the future.

The trustees announce with much pleasure that the plans looking towards the construction of a new and permanent laboratory building have in the past year made substantial progress. At the Baltimore meeting, in December, 1908, was announced a gift from the President of the Board of Trustees, Mr. Charles R. Crane, for the purchase of additional land at Woods Hole with a view to the future erection of such a laboratory building. With the approval of the donor, the fund thus acquired was used for the purchase of the tract known as the "Kidder lot," lying between the stone building and the street on which the original laboratory building faces. At the August meeting in 1909 Mr. Coolidge offered to prepare a tentative plan for a permanent fire-proof building, to be erected on this site, and a committee of the trustees was appointed to secure suggestions and details incident to the preparation of this plan, and to coöperate with the Corporation committee on building. At the Boston meeting, in December, 1909, a new committee was appointed to carry on this work, and it is hoped that during the present year the plans may take definite shape.

The time has come when the laboratory should be provided with a building or buildings commensurate with the extent and

importance of the work in which it is engaged, and which will not suffer by comparison with the laboratories of other institutions of similar aim. The steady expansion of our work in past years has from time to time made imperative the construction of new buildings; and that these have fulfilled their purpose is abundantly demonstrated by the work that has been done in them. Owing to financial limitations, however, most of these buildings were of a flimsy and temporary character, sometimes erected in haste to meet an immediate and pressing need; and they are not only in themselves imperfectly adapted to their purpose but have often called forth expressions of astonishment from foreign and other visitors that so much could have been accomplished with accommodations so primitive. The cramped and often noisy working quarters, the unsteady foundations, imperfect equipment and inflammable nature of these buildings impress every worker and visitor at the laboratory; and nothing is plainer than the need of a substantial, well equipped, safe and dignified new laboratory to form the principal center of the work at Woods Hole. The acquisition of a suitable site for this purpose is an important forward step, and the generous offer of Mr. Coolidge places the project before us in concrete form. It is the earnest hope of the trustees that the means may soon be found for carrying out the plans that are now being formulated; and it is their belief that when this has been done the laboratory will enter upon a new period of development that contains great possibilities of usefulness.

V. TREASURER'S REPORT

FOR THE YEAR ENDING DECEMBER 31, 1909

INCOME

Annual dues	\$	666.00	
Donations		17,240.00	
Miscellaneous:			
Interest on deposits.....	\$44.40		
Bath house.....	78.45		
Mess (net).....	26.74		
Rent of microscopes.....	49.00		
Sundries	6.85	205.44	
Supply Department		8,549.55	
Tuitions		3,708.35	\$30,369.34

EXPENSES

Administration	\$	900.00	
Advertising		80.72	
Biological Bulletin (net).....		1,094.69	
Boats and launch.....		1,595.92	
Chemicals, chemist supplies, etc.		659.32	
Fish trap (net).....		113.16	
Instructors' salaries		2,725.00	
Insurance		98.78	
Interest		150.00	
Labor		3,566.58	
Periodicals and library supplies.....		868.39	
Postage		79.24	
Real estate		7,600.00	
Repairs		1,011.21	
Sundries		2,262.09	
Supply department		6,380.69	\$29,185.79
Surplus			\$ 1,183.55

ITEMIZED LIST OF SUNDRY EXPENSES FOR THE YEAR 1909

Announcements	\$ 69.00	
Care of cemetery lot.....	2.00	
Cleaning	35.00	
Crash	50.45	
Dr. Drew's expenses.....	157.49	
Dr. Lillie's expenses.....	371.35	
Engrossing Woods Hole greetings.....	20.00	
Exchange on checks.....	32.68	
Expenses W. C. Curtis.....	44.85	
Expenses purchase of land at Woods Hole...	10.31	
Express	158.95	
Gardener	4.70	
Sundry supplies	169.06	
Hubbard, J. G., services.....	60.00	
Instruments, laboratory supplies.....	131.15	
Lamps	13.85	
Laundry	17.29	
Lecture expense	25.00	
Plowing	3.00	
Premium on bond.....	7.50	
Rent of microscopes.....	44.16	
Rent of typewriter.....	11.50	
Stationary, office supplies.....	129.40	
Sundries	32.43	
Teaming and livery.....	14.00	
Telephone	61.97	
Treasurer's office, clerical services.....	520.00	
Water	65.00	\$2,262.09

MARINE BIOLOGICAL LABORATORY INVESTMENTS

JANUARY 1, 1910

RESERVE FUND

Amount of fund December 1, 1899.....	\$4,553.14	
Received from life memberships	600.00	
Income to January 1, 1910.....	1,898.15	
Gain from sale of securities.....	372.20	
	7,423.49	
Paid for current expenses of laboratory.....	6,000.00	\$1,423.49
Reserve fund now consists of the following:		
\$3000 Am. Tel. & Tel. Co. 4s, cost.....	2,921.25	
6 shs. Am. Smelting & Refining Co. pfd.....	732.00	
Cash	770.24	
	4,423.49	
The above stocks and bonds are held as collateral		
for loan of.....	3,000.00	1,423.49

LIBRARY FUND

Amount of fund December 1, 1899.....	866.15	
Income	626.47	
Gain from sale of securities.....	74.88	
Sale of rights.....	.81	1,568.31

Library Fund now consists of the following:

3 shs. Am. Tel. & Tel. Co., cost.....	383.25	
½ of \$1000 Am. Tel. & Tel. Co. 4s, cost....	779.00	
1 sh. Am. Smelting & Refining Co. Pfd., cost	122.00	
Cash	284.06	1,568.31

LUCRETIA CROCKER FUND

Amount of fund December 1, 1899.....	2,500.00	
Income after paying students' fees.....	488.91	
Sale of rights.....	.94	2,989.85

TREASURER'S REPORT.

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Lucretia Crocker Fund now consists of the following:

18 shs. Vermont & Mass. R. R. Co., cost....	2,416.50	
1 sh. West End St. Ry. Co., cost.....	83.00	
1 sh. Am. Tel. & Tel. Co., cost.....	127.75	
$\frac{1}{8}$ of \$1000 Am. Tel. & Tel. Co., 4s, cost.....	194.75	
Cash	<u>167.85</u>	2,989.85

REPORTS OF THE TREASURER,
1906 TO 1909

SUMMARY OF THE REPORTS OF THE TREASURER
OF THE MARINE BIOLOGICAL LABORATORY
JANUARY 1, 1907, TO JANUARY 1, 1910

RECEIPTS

Cash on hand January 1, 1907.....	\$	1.19
Income for 1907.....	15,779.70	
Income for 1908.....	17,452.18	
Income for 1909.....	30,369.34	\$63,602.41

DISBURSEMENTS

Expenses for 1907.....	\$15,638.38	
Expenses for 1908.....	17,476.25	
Expenses for 1909.....	29,185.79	
Cash on hand December 27, 1909.....	1,301.99	\$63,602.41

VI. THE DIRECTOR'S REPORT

TO THE TRUSTEES OF THE MARINE BIOLOGICAL LABORATORY.

Gentlemen. I have the honor to submit herewith a report on the work of the twenty-second session of the Marine Biological Laboratory for the year 1909. The year has been a successful one as measured by the growth of all departments, by the genuine and hearty coöperation of naturalists and institutions, and by the increase in our resources. If any confirmation were needed of the necessity and value of the work of the Laboratory, this year has furnished it.

Trustees.—At the regular summer meeting of the corporation, the following new members were elected to the board of trustees: Nathaniel L. Britton, Director-in-Chief of the New York Botanical Garden, Alfred G. Mayer, Director of the Marine Laboratory of the Carnegie Institution, and George Lefevre, Professor of Zoölogy in the University of Missouri. The board is to be congratulated on the accession of these gentlemen to its membership, which is now complete, consisting of thirty-two regular members and four members *ex officio*.

Staff.—The staff of investigation and instruction in 1909 comprised twenty-seven members, representing seventeen universities and colleges. There was no change in heads of departments; but a new course was offered by Professor Edward G. Spaulding on "Philosophical Aspects of Biology and Allied Sciences," which aroused much interest. The members of the staff of investigation rendered their services free.

The work of organizing the library on a more effective basis received a great impetus owing to the efforts of the librarian, Dr. Knowler. It is becoming increasingly important that more funds should be devoted to the purchase of books. There is no question that the enlargement of our library will greatly strengthen our position with the scientific public.

Attendance.—The total attendance in 1909 was 129 as against 100 in 1908. Of this number 66 were investigators and 63 stu-

dents. The gain was divided almost equally between investigators and students, fourteen of the former and fifteen of the latter. The class of beginning investigators alone showed a gain of 10, 16 in 1909 as against 6 in 1908. This was one of the most encouraging features of the session. We should continue our efforts to increase the attendance of this class of workers, because it is one of the most important functions of our Laboratory to assist in the making of investigators; and because those who are to succeed us in the work of advancing our science and in the management of the Laboratory are represented by the younger investigators of to-day.

Subscribing Institutions.—The number of subscribing institutions was 19 in 1909 as against 17 in 1908; Princeton University, Oberlin College, and the University of Missouri were new, and we hope permanent, accessions. We are still, however, far from having reached the limit of growth in this respect and we may confidently expect new additions. A committee of the board is now engaged in correspondence with institutions on this subject.

Donations.—The president of the board has again provided for the deficit in running expenses. He has also presented the Laboratory with a large piece of land having 157 feet of frontage on the main street and more than twice that on East Street, which unites our two main holdings and forms an ideal site for the erection of future permanent buildings.

Supply Department.—The business of the Supply Department continues to grow in a satisfactory manner; it has been necessary to furnish Mr. Gray with two assistant collectors during the year instead of one as formerly, and a bookkeeper is also employed throughout the year, but the growth of the business has been more than sufficient to pay for the additional expense involved.

In conclusion, I wish to express my thanks to the board of trustees for their hearty support and to the members of the staff for their loyal coöperation in the work of the Laboratory.

There are appended as parts of this report (1) a list of the staff, (2) the names of subscribing institutions, (3) lists of investigators and students in attendance, (4) a comparative tabular view of attendance, (5) the evening lectures, (6) a list of members of the Corporation.

THE STAFF, 1909

F. R. LILLIE, DIRECTOR,
Professor of Embryology, University of Chicago.

GILMAN A. DREW, ASSISTANT DIRECTOR,
Professor of Biology, University of Maine.

ZOÖLOGY

I. INVESTIGATION

Zoölogy and Embryology

- E. G. CONKLIN.....Professor of Zoölogy, Princeton University.
GILMAN A. DREW.....Professor of Biology, University of Maine.
ROSS G. HARRISON.....Professor of Comparative Anatomy, Yale University.
GEORGE LEFEVRE.....Professor of Zoölogy, University of Missouri.
WARREN H. LEWIS.....Associate Professor of Anatomy, Johns Hopkins University.
FRANK R. LILLIE.....Professor of Embryology, University of Chicago.
THOS. H. MONTGOMERY, JR.....Professor of Zoölogy, University of Pennsylvania.
E. B. WILSON.....Professor of Zoölogy, Columbia University.
(Absent in 1909.)

II. INSTRUCTION

- WINTERTON C. CURTIS...Professor of Zoölogy, University of Missouri.
GIDEON S. DODDS.....Fellow in Zoölogy, University of Pennsylvania.
A. S. PEARSE.....Instructor in Zoölogy, University of Michigan.
JOHN W. SCOTT.....Westport High School, Kansas City.

HENRY J. SPENCER Assistant in Zoölogy, Columbia University.
 EDWARD E. WILDMAN.... Central High School, Philadelphia.

EMBRYOLOGY

I. INVESTIGATION. (See Zoölogy)

II. INSTRUCTION

GILMAN A. DREW..... Professor of Biology, University of Maine.
 LORANDE L. WOODRUFF... Assistant Professor of Biology, Yale University.
 WILLIAM E. KELLICOTT.. Professor of Biology, Woman's College of Baltimore.
 ROBERT A. BUDINGTON... Associate Professor of Zoölogy, Oberlin College.

PHYSIOLOGY

I. INVESTIGATION

ALBERT P. MATHEWS.... Professor of Physiological Chemistry, University of Chicago.
 R. S. LILLIE..... Instructor in Comparative Physiology, University of Pennsylvania.

II. INSTRUCTION

H. H. NEWMAN..... Professor of Zoölogy, University of Texas.
 FRANK P. KNOWLTON.... Professor of Physiology, Syracuse University.
 F. H. PIKE..... Instructor in Physiology, University of Chicago.

PHILOSOPHICAL ASPECTS OF BIOLOGY AND ALLIED SCIENCES

LECTURES

EDWARD G. SPAULDING... Assistant Professor of Philosophy, Princeton University.

BOTANY

GEORGE T. MOORE..... Professor of Plant Physiology and Applied Botany, Washington University.
 HENRY KRAEMER..... Professor of Botany and Pharmacognosy, Philadelphia College of Pharmacy.
 M. B. THOMAS..... Professor of Botany, Wabash College.
 GEORGE R. LYMAN..... Assistant Professor of Botany, Dartmouth College.

LIBRARY

H. McE. KNOWER.....Associate in Anatomy, Johns Hopkins University, Librarian.

OLIVER S. STRONG.....Instructor in Anatomy, Columbia University, Chemist

G. M. GRAY.....Curator of Supply Department.

JACOB SCHRAMM.....Wabash College, Collector in Botany.

JOHN VEEDER.....Cockswain.

INVESTIGATORS

1909

OCCUPYING ROOMS

1. ZOÖLOGY

- ADDISON, W. H. F., University of Pennsylvania.
BECKWITH, CORA J., Instructor in Biology, Vassar College.
BENTLEY, MADISON, Assistant Professor of Psychology, Cornell University.
BUDINGTON, ROBERT A., Associate Professor of Zoölogy, Oberlin College.
CLARK, ELIOT R., Instructor in Anatomy, Johns Hopkins Medical School.
CONKLIN, E. G., Professor of Zoölogy, Princeton University.
CURTIS, W. C., Professor of Zoölogy, University of Missouri.
DODDS, GIDEON S., Fellow in Zoölogy, University of Pennsylvania.
DREW, GILMAN A., Professor of Biology, University of Maine.
GOLDFARB, A. J., Investigator, Columbia University.
GUDERNATSCH, J. F., Instructor in Embryology and Experimental Morphology,
Cornell University Medical School, New York City.
HARRISON, ROSS G., Bronson Professor of Comparative Anatomy, Yale University.
HARVEY, B. C. H., Assistant Professor of Anatomy, University of Chicago.
KELLCOTT, WILLIAM E., Professor of Biology, Woman's College, Baltimore.
KING, HELEN D., Assistant in Anatomy, Wistar Institute of Anatomy and
and Biology.
KIRKHAM, WILLIAM B., Instructor in Biology, Yale University.
KNOWER, H. McE., University of Toronto.
LEFEVRE, GEORGE, Professor of Zoölogy, University of Missouri.
LILLIE, FRANK R., Professor of Embryology, University of Chicago.
LEWIS, WARREN H., Associate Professor of Anatomy, Johns Hopkins University.
McDONOUGH, JAMES, Decatur Ill.
MCGILL, CAROLINE, Instructor in Anatomy, University of Missouri.
MAYER, ALFRED G., Carnegie Institution, Washington, D. C.
MONTGOMERY, THOS. H., JR., Professor of Zoölogy, University of Pennsylvania.
MORGAN, T. H., Professor of Experimental Zoölogy, Columbia University.
MORRILL, C. V., Lecturer in Histology and Embryology, Syracuse University.
MURBACH, LOUIS, Central High School, Detroit, Mich.
PAYNE, FERNANDUS, University of Indiana.
PEARSE, A. S., Instructor in Zoölogy, University of Michigan.
QUACKENBUSH, L. S., Columbia University.
RICKETTS, H. T., Assistant Professor of Pathology, University of Chicago.
SCOTT, JOHN W., Westport High School, Kansas City, Mo.
SPENCER, HENRY J., Assistant in Zoology, Columbia University.
STOCKARD, CHARLES R., Assistant Professor of Embryology and Comparative
Anatomy, Cornell Medical School.

STRONG, OLIVER S., Instructor in Anatomy, Columbia University.
WILDMAN, EDWARD E., Central High School, Philadelphia, Pa.
WILSON, E. B., Professor of Zoölogy, Columbia University.
WOODRUFF, L. L., Assistant Professor of Biology, Yale University.
YERKES, ROBERT M., Professor of Comparative Psychology, Harvard University.

2. PHYSIOLOGY

BRADLEY, HAROLD C., Assistant Professor of Physiological Chemistry, University of Wisconsin.
BUNZEL, H. H., University of Chicago.
KNOWLTON, FRANK P., Professor of Physiology, Syracuse University.
LILLIE, R. S., Instructor in Physiological Zoölogy, University of Pennsylvania.
MATHEWS, ALBERT P., Professor of Physiological Chemistry, University of Chicago.
NEWMAN, H. H., Professor of Zoölogy, University of Texas.
PIKE, FRANK H., Instructor in Physiology, University of Chicago.

3. BOTANY

KRAEMER, HENRY, Professor of Botany and Pharmacognosy, Philadelphia College of Pharmacy.
LYMAN, GEORGE R., Assistant Professor of Botany, Dartmouth College.
MOORE, GEORGE T., Professor of Plant Physiology and Applied Botany, Washington University, St. Louis.
THOMAS, MASON B., Professor of Botany, Wabash College.

OCCUPYING TABLES

1. ZOOLOGY

BOONE, W. D., University of Missouri.
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1909

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4. SCHOONOVER, ELIZABETH H., Smith College.
5. SPALDING, CAROLINE, Wellesley College.
6. TAYLOR, ARTHUR W., Teacher, Salem, Mass.

TABULAR VIEW OF ATTENDANCE

	1907	1908	1909
INVESTIGATORS—Total	60	52	66
Occupying Rooms			
Zoölogy	32	32	39
Physiology	8	6	7
Botany	10	8	4
Occupying Tables			
Zoölogy	9	6	14
Physiology	1		2
STUDENTS—Total	47	48	63
Zoölogy	34	19	36
Embryology		15	12
Physiology	4	3	9
Botany	9	11	6
UNIVERSITIES AND COLLEGES REPRESENTED.	47	51	47
Investigators	26	25	27
Students	21	26	20
SCHOOLS REPRESENTED	13	5	14
Investigators	4	2	3
Students	9	3	11

SUBSCRIBING INSTITUTIONS, 1909

ACADEMY OF NATURAL SCIENCES, PHILADELPHIA.
MOUNT HOLYOKE COLLEGE.
ROCHESTER UNIVERSITY.
SMITH COLLEGE.
UNIVERSITY OF CHICAGO.
COLUMBIA UNIVERSITY.
UNIVERSITY OF PENNSYLVANIA.
VASSAR COLLEGE.
UNIVERSITY OF MICHIGAN.
WELLESLEY COLLEGE.
UNIVERSITY OF MISSOURI.
WISTAR INSTITUTE OF ANATOMY AND BIOLOGY.
WOMAN'S COLLEGE OF BALTIMORE.
YALE UNIVERSITY.
PRINCETON UNIVERSITY.
VASSAR BROTHERS' INSTITUTE.
NORTHWESTERN UNIVERSITY.
OBERLIN COLLEGE.
UNIVERSITY OF KANSAS WOMAN'S TABLE SUPPORTED BY MRS.
ROBINSON.
LUCRETIA CROCKER SCHOLARSHIP, BOSTON PUBLIC SCHOOLS.

EVENING LECTURES, 1909

- T. H. MORGAN....." Mendelian Inheritance of Coat and
Eye Color in Mice " July 6.
- A. P. MATHEWS....." The Mechanism of Some Proto-
plasmic Combustions " July 9.
- ROSS G. HARRISON....." Protoplasmic Movement as a Fac-
tor in the Development of the
Nervous System " July 13.
- C. B. DAVENPORT....." Variation in Dominance " July 16.
- HENRY KRAEMER....." Experiments on the Modification
of Color in Plants "..... July 23.
- T. H. MONTGOMERY, JR..." Architectural Habits of Spiders " July 27.
- W. L. TOWER....." The Role of External Factors in
Variation and Heredity " July 29.
- H. H. NEWMAN....." A Unique Form of Reproduction
in the Nine-banded Armadillo " July 30.
- M. M. METCALF....." The Protozoan Nucleus " Aug. 6.
- C. H. EIGENMANN....." An Expedition to British Guiana
and the Kaieteur " Aug. 9.
- G. H. PARKER....." Sponge Reactions and the Origin
of the Nervous System " Aug. 11.

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OVULATION IN MAMMALS, WITH SPECIAL REFERENCE TO THE MOUSE AND RAT.

WILLIAM B. KIRKHAM.

Ovulation and its connection, if any, with pairing is a subject that has long engaged the attention of mammalian embryologists. As long ago as 1828 Von Baer made the statement, seemingly based more upon theoretical than upon experimental grounds, that the Graafian follicles of mammals appear not to rupture without pairing, or some analogous stimulation of the female sexual organs. It would seem to be a simple matter to determine in a given mammal whether ovulation was, or was not dependent upon copulation, but the literature of the subject shows long controversies about this point. The matter can best be taken up by considering individually the animals thus far studied. The egg of the dog was carefully investigated by Bischoff ('45), who declared that in that animal ovulation is entirely independent of pairing. This observation has since been confirmed by Marshall and Jolly ('05), who also found that the bitch has typically two sexual seasons each year. Further confirmation has come from Ancel and Bouin ('08), who, in addition to finding ovulation occurring independently of copulation, discovered that the Graafian follicles in the dog rupture in turn.

Ovulation phenomena in the guinea-pig gave rise to a spirited controversy between Bischoff ('52 and '66), who claimed that the Graafian follicles in that animal ruptured entirely independently of pairing, and Reichert ('61), who declared that ovulation only occurred nine to ten hours after copulation had taken place. In his second paper, replying to Reichert, Bischoff states that he has experimentally demonstrated in dogs, rabbits, guinea-pigs, rats, sheep and swine that follicles mature and rupture without relation to pairing, the full data of this work being given in an earlier paper (Bischoff '44). Hensen ('76), in his paper on the early development of the guinea-pig, quotes the opposing views of Bischoff and Reichert, without expressing any opinion

of his own, and Lams and Doorme ('07) quote Reichert alone. The careful researches of Rubaschkin ('05), however, have definitely confirmed Bischoff's original contention, and it may therefore be considered as finally settled that in the guinea-pig ovulation and pairing are essentially independent phenomena.

The rabbit's egg has been studied by Barry ('39), Bischoff ('42), Coste ('47), Heap ('05), and by Dubreuil and Regaud ('08). Barry, and the last three authors named above find, contrary to Bischoff, that the rabbit cannot ovulate until a short time after pairing has occurred, but Coste claims to have induced ovulation by taking females which, isolated from males, had been in heat for a number of days, and allowing males to cover them without, however, allowing them to remain together long enough for actual insemination to take place. Heape, and Dubreuil and Regaud, on the contrary, find that if, in this animal, pairing for any reason does not occur during the oestrus period, the eggs which would naturally have been discharged all degenerate within the ovaries. Indeed, according to Heape, if the male is withheld from the doe during several consecutive periods of oestrus, most, if not all, of the older and many of the younger follicles undergo degeneration, and this may result in a more or less permanent sterility.

Marshall ('03 and '04) has studied the oestrus cycle in the sheep, and in the common ferret. He states that Scotch black-faced ewes can, during the regular sexual season, ovulate without pairing, as claimed by Bischoff ('44) for ewes in general, but pairing, according to Marshall, even then may hasten the process of ovulation, which outside of such rutting periods may be dependent upon copulation. The common ferret, Marshall finds, behaves as recent investigators have found to be the case with the rabbit, ovulating only after pairing. The withholding of the male greatly prolongs the period of heat, and in a few exceptional cases is said to lead to the death of the female.

Benecke ('79), Eimer ('79), Fries ('79), Van Beneden and Julin ('80), and Van Beneden ('99) are all agreed that in all the various species of bats that they have studied, pairing takes place several weeks before the eggs leave the ovaries.

The condition of affairs in mice has been disputed by Sobotta ('95 and '07) and Kirkham ('07), who have claimed the inde-

pendent occurrence of ovulation and pairing, by Gerlach ('06), who denied such independence, and by Lams and Doorme ('07), who state that ovulation in the mouse is perhaps connected with pairing.

Bischoff ('44) did a little work with rats, and he came to the conclusion that in them ovulation could take place without pairing, but he confessed that his evidence was incomplete.

In view of these uncertain and conflicting statements, it has seemed desirable to submit the matter of the relation, if any, between ovulation and pairing, to a critical test in the case of the rat and the mouse, using, in some instances, individuals that had been isolated from as early a period as the sex could with certainty be externally determined. The four female white mice used were virgins from two litters, by different parents, and were kept apart from all males, including their brothers, from the time they were two weeks old. Three of these females were born in the laboratory in the middle of December, 1908, and the other early in January, 1909, and all four, until they had been weaned, were isolated with their respective mothers.

One of the virgins born the previous December was chloroformed on March 19, 1909, a day selected by chance, without any special calculation other than the general knowledge that the mice born about the same time as the test animals were beginning to show signs of pregnancy. When the body of this animal was opened, and the ovaries examined, bright red spots were found upon both these organs, a characteristic sign that ovulation had recently occurred. The ovaries and Fallopian tubes were then removed from the body, and serial sections were prepared in a manner described in a previous paper (Kirkham '07). The study of this material has revealed seven eggs in the Fallopian tubes, beside numerous eggs still in the ovaries, the latter undergoing degenerative changes after having formed the first polar body and second polar spindle. The tube eggs appear in every respect like those obtained from females which had paired, and were then killed before the spermatozoa had reached the eggs. A varying number of follicle cells are found associated with the eggs, the zona pellucida in all cases is sharply defined, all the eggs possess second polar spindles, and in two instances the first polar body is still visible, although decidedly atrophied.

The remaining three virgin mice were killed March 30, 1909, and of those born in the preceding December, the Fallopian tubes of one yielded nine eggs, similar to those mentioned above, except that seven still showed first polar bodies; the ovaries of the other animal had upon them the white scars indicative of well formed corpora lutea, and no sign of eggs was found in either Fallopian tube. Both sets of ovaries contained a number of mature eggs undergoing degeneration. The female mouse born in January showed no signs of having ovulated, no evidence of either young or old corpora lutea being found on either ovary, nor any trace of eggs in the Fallopian tubes. The ovaries of this animal, however, have in them several eggs with first polar bodies and second polar spindles, but all appear to be degenerating.

From these observations we may safely conclude that not only is ovulation in adult female white mice independent of pairing, as stated by Sobotta ('95 and '07), and by the writer ('07), but that it is an independent process from the start, maturation and ovulation occurring in the females without regard to pairing, although at present we do not know with what regularity.

These results, also, are not only of general interest, but they possess value as showing the mouse to be an eminently suitable mammal upon which to investigate the possibility of artificial parthenogenesis.

A further fact which should be noted here, is the change of opinion, on the part of the writer, regarding the fate of the first polar body formed by the mouse egg. In 1907 the writer stated that his observations led him to believe that when the first polar body was not found with the egg, it had been forced through the zona pellucida at the time of ovulation. That same year Lams and Doorme ('07) published a paper on the development of the mouse egg in which they stated that the first polar body in that animal underwent degenerative changes leading finally to its disappearance. These authors figured a few first polar bodies in an advanced state of atrophy, and the present writer has since confirmed their conclusions, using some material of his own obtained since the publication of his first statement on the subject. A fairly complete series of degenerating first polar bodies — some

belonging to eggs themselves degenerating within the ovaries, others in the process of early cleavage in the Fallopian tubes—make it practically certain that in the case of such mouse eggs as have reached or passed the stage when the second maturation spindle is formed, and yet show no trace of the first polar body, this body has broken up and disappeared; in all instances of such degeneration the chromatin granules lose their distinctive staining qualities some time before the final dissolution of the polar body cytoplasm.

The observations on rats were limited to two adult white females. These animals were kept in a large cage with males and other females until they showed signs of advanced pregnancy. They were then isolated in separate cages, and a close watch was kept for the appearance of the litters. As soon as found, the young rats were taken from their mothers, and then the latter were killed, one twenty-four, the other forty-eight hours later; it having been previously determined that the white rat, like the white mouse, during the warm months of the year is usually in heat directly after giving birth to a litter. When the bodies of these animals were opened, characteristic corpora lutea were visible on all the ovaries, and after the ovaries and Fallopian tubes had been sectioned and stained, eight tube eggs were found in one animal, and five in the other. These eggs have exactly the appearance of eggs obtained from female white rats which have been allowed to pair and then are killed just before fertilization can take place; the zona pellucida in all cases is well preserved, all of the eggs possess second polar spindles, while a majority show no trace of the first polar body, it probably having previously degenerated.

The points to be emphasized in these researches are three in number:

1. Virgin white mice mature eggs and discharge them from their ovaries at practically the same age as their sisters who have been allowed to pair, and such eggs show no differences from other unfertilized eggs.

2. Adult female white rats during the active breeding season ovulate regularly, immediately after giving birth to a litter, whether pairing is or is not allowed to take place.

3. The first polar body has degenerated in such mouse and rat eggs as show no trace of it, and yet have reached or passed the stage of the second maturation spindle.

SHEFFIELD SCIENTIFIC SCHOOL OF YALE UNIVERSITY,
March, 1910.

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DESCRIPTION OF AN ABNORMAL LOBSTER CHELIPED.¹

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While visiting the United States Fish Hatchery at Gloucester, Mass., during the summer of 1908, my attention was called to an abnormal lobster (*Homarus americanus*) which was at that time preserved in formalin. The specimen was taken in Gloucester harbor by a local fisherman in the preceding May, and was kept alive in an aquarium at the hatchery about a week before it died. Through the kindness of Mr. Corliss, superintendent of the hatchery, I was enabled to secure the specimen for description. The abnormality consists of a double extra claw on the left cheliped, and while not different in general type from specimens which have previously been described, it does possess certain peculiarities which would appear to justify placing it on record. The right cheliped was missing when the lobster was caught. From the appearance of the scar, and the fact that there is no evidence of regeneration having begun, it is probable that the loss of the appendage was comparatively recent. The especial interest of the present specimen lies in the fact that the extra claws are so well developed and that their arrangement is almost schematic of the principles, or "rules" of secondary symmetry laid down by Bateson (1894, p. 479), if we make allowance for the torsion which normally occurs in the cheliped of the lobster.

DESCRIPTION.

The lobster possessing this abnormality is a male, 237 mm. in length from tip of rostrum to end of telson.

Claws.—The part which represents the primary claw (*PrL* of figures) is practically a normal left chela of the *crushing* type, except for an abnormal outgrowth from its outer (morpho-

¹ Paper from the Zoological Laboratory, Sheffield Scientific School of Yale University.

logically posterior) face. This outgrowth does not arise from the middle of the outside face of the primary propodus, but rather from the ventral half of its surface, the significance of which in its relation to the planes assumed by the various claws will be discussed further on. In general appearance the outgrowth represents a more or less completely doubled extra claw,¹ and according to the classification given by Emmel (1907) would fall in the class having "abnormal processes arising from the normal propodite," and under this heading it would probably have to be classed with those having "two extra indices and double extra dactyl." It differs, however, from any previously described specimens in this category, so far as I am aware, in the degree to which the double dactyl approaches the condition of two separate dactyls, it being markedly bifurcated at the tip, whereas in the case (specimen No. 4) described by Emmel, what he takes to be the double dactyl is only a small undivided "stump." The strength of his interpretation lies in the fact that both the double dactyl and the index in other specimens may be represented by an undivided process, the double character of which is indicated by its having teeth on two opposite sides (cf. Emmel's specimens No. 2 and No. 3).

Considering now only the supernumerary outgrowth, the basal part, which represents a double propodus, has along its ventral side a deep groove, as may be seen in Fig. 2. The continuation of the divergent ridges which bound this groove form two separate and distinct indices ($I'R$ and $I'L$), each of which, taken by itself, appears like a practically normal index. They are, moreover, essentially alike except that their relations of symmetry are reversed, so that one would appear as the image of the other in a plane mirror placed between them. This relation will, however, be discussed more fully later. The opposing dactyl is also divided for nearly half its length, on account of the bifurcation mentioned above, the divergent branches ($D'R$ and $D'L$) meeting in opposition the corresponding extra indices ($I'R$ and $I'L$). The double character of the dactyl clear to its base is indicated by the fact that the rows of teeth on the two tips continue in separate and distinct lines down the inside of the basal portion. Further-

¹ The extra claws are somewhat smaller than the normal or primary claw.

more, on the dorsal side near the base, the single spine of the primary dactyl (*SpDL*) is represented by two similar spines (*SpD'R* and *SpD'L*). The row of spines down the dorsal ridge of the extra propodite is very weak, whereas there is a row of strong spines in the corresponding position on the normal propodite (*PrL*). This is a result of the "compounding" at this level, to be mentioned again below (p. 264).



FIG. 1. Photograph of abnormal cheliped from dorsal side. (For explanation of abbreviations used in the figures see list on p. 267.)

The distance between the tips of the two diverging extra dactyls (*D'R* and *D'L*) is very slightly greater than that between the tips of the two extra indices (*I'R* and *I'L*), so that if the double dactyl closed down squarely it would tend to make the tips lap

over on the outside (morphologically dorsal side) of each of the index tips; but, as may be seen in Fig. 1, they do not close squarely, but instead the double claw shuts in such a manner that *both the dactyl tips overlap the index tips on the side toward the normal (primary) claw*. Thus $D'L$ overlaps on the *ventral* side of $I'L$, and $D'R$ on the *dorsal* side of $I'R$. In this connection it

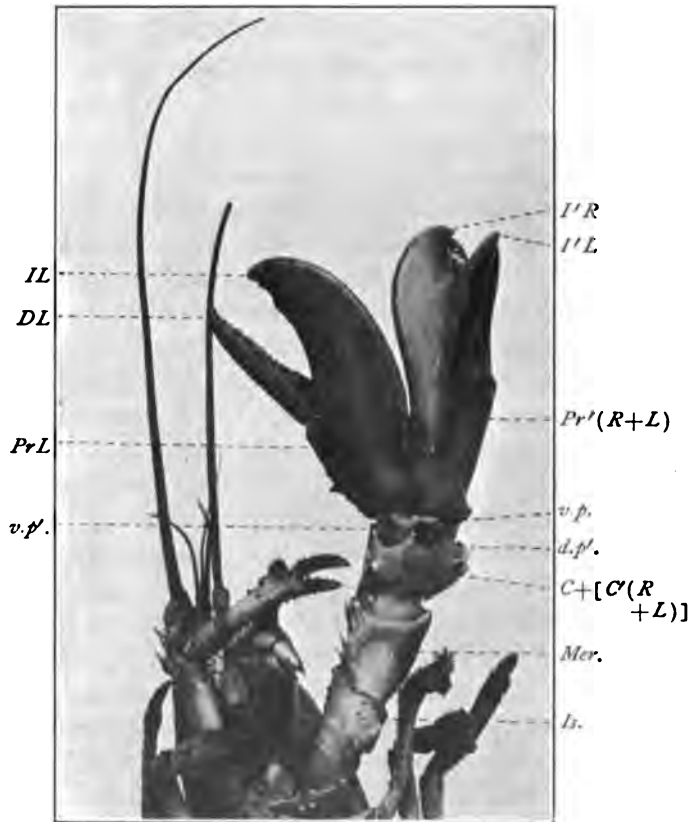


FIG. 2. Photograph of abnormal cheliped from ventral side.

is interesting to note that the normal dactyl (DL) when closed overlaps decidedly on the ventral side of its index (IL), that is, on the side away from the extra claws. All these dactyls thus overlap their respective indices on the side toward the median plane of the animal, in this matter not agreeing with the morpho-

logical symmetry which obtains in other respects. As is to be expected the teeth on the extra claws are plainly, like those of the normal claw, of the "crusher" type.

No attempt was made to study the internal anatomy further than to note that the extra double dactyl has *two* adductor tendons, but only a single abductor tendon. This condition is what might be expected from the way in which the two extra dactyls (*D'R* and *D'L*) are "compounded" (*vide infra*) at their base.

Carpopodite.—The effect of the doubled condition distally is very evident in the carpopodite. It is much broader distally than a normal carpopodite, especially as viewed from above. This is due in large part to an extra process (*d.p'*) on the posterior face of the appendage in the middle of the area which is evenly concave in the normal lobster. This process is in line with the double dactyl (*D'L* and *D'R*), thus having the same relations to this doubled extra part that the dorsal articular process (*d.p.*) has to a normal dactyl. Opposite *d.p'*, in the concavity of the anterior side, is a smaller process (*v.p'*). The normal ventral articular process (*v.p.*) is present, but, apparently due to the disturbing influence of the abnormal parts, the articulation of the chela with it is not close.¹ The only close articulation is, then, with the dorsal process (*d.p.*), and although the chela can be moved on this in the normal plane of movement, it is capable of a slight movement in other directions as well. The hinge-like movement in the normal plane (*i. e.*, on the axis between *d.p.* and *v.p.*) is, moreover, greatly limited by the secondary processes (*d.p'* and *v.p'*). Whereas in the normal lobster the claw (propodite) can swing on the carpopodite through an arc of about 90°, it is limited here to a movement of 25°. A smooth spot on the shell (*x*, Fig. 3) shows where *d.p'* has stopped the movement of the claw in that direction, and the nature of the surfaces at *v.p.* shows that in the living animal there must have been some movement of the claw in the plane at right angles to the normal plane of movement.

¹ It would look as though the presence of muscles belonging to the abnormal part, which by their action tended to swing the claw on the secondary axis *d.p'-v.p'*, had prevented a close articulation at *v.p.* A careful study of the musculature of the carpopodite would be of much interest, but it was found impracticable to make such a study at the present time.

The arrangement of certain of the spines also goes to show that *d.p'*. is to be considered as the equivalent of a double dorsal articular process corresponding to the doubled abnormal claw; but there is no need to go into the details of this matter, which can be made out in the figures.

Meropodite.—Here, too, the abnormality is evident. The joint is relatively much thicker than the corresponding joint in a

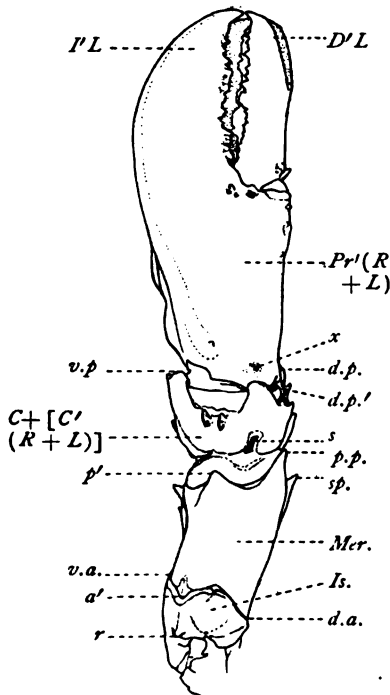


FIG. 3. Abnormal cheliped seen from posterior side (really a little ventro-posterior).

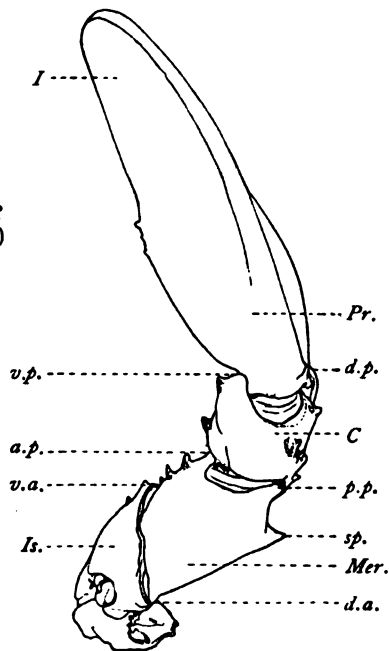


FIG. 4. Normal left cheliped of a lobster, seen from posterior side (really a little ventro-posterior).

normal lobster. The normal plane of articulation between the meropodite and the carpodite is morphologically horizontal, the articular processes therefore being anterior (Figs. 3 and 4, *a.p.*) and posterior (*p.p.*). These processes and the articulation are essentially normal in the abnormal claw, but in the concavity of the (morphologically) ventral side is a large process (*p'*) which apparently represents one point for an incipient axis of articu-

lation in a plane at right angles, or nearly so, to the normal articulation. This condition corresponds to that between the carpopodite and the propodite, described above. There is no evidence of the corresponding process on the opposite side, however, nor is there any articulation with p' , the distal portion swinging normally on the axis between $a.p.$ and $p.p.$ But while this movement covers an arc of approximately 45° in a normal claw, it is here limited to a range of between 25° and 30° . There is no sign of duplication of the large spine ($sp.$) on the dorsal side of the meropodite.

Ischiopodite.—The ischiopodite, instead of being broad and flat, as in normal cases, is broadly triangular in cross section, while the line of articulation between it and the meropodite is much less oblique than normal. The motion at this place is normally (in the legs following the cheliped) on an essentially dorso-ventral axis ($d.a.-v.a.$), though due to torsion in the chelipeds it becomes greatly inclined. In the abnormal specimen the dorsal articulation ($d.a.$) appears practically normal, but on the ventral side there are two points of articulation ($v.a.$ and a'). Being thus hinged at three places the joint is stiff and incapable of movement in any plane. The row of spines running down the ventral side of the meropodite and continued on the ischiopodite, proves the articulation ($v.a.$) in line with these to represent the normal one, while the other (a') is secondary.

The articulation between the ischiopodite and the basal podomeres is apparently normal, though the ischiopodite here has on its posterior side a ridge (r) which is not present in normal specimens. This ridge ends in a short, blunt process, much like an articular process, but there is no articulation with it. It does in all probability, however, represent such a secondary process, being placed, as in the other joints, midway between the normal points of articulation.

RELATIONS OF SYMMETRY.

Torsion.—It is a matter of common knowledge that in development the cheliped of the lobster undergoes a twisting or torsion, as a result of which the chela come to lie in a nearly horizontal plane, that is, almost at right angles to their normal

morphological plane. This is illustrated in Fig. 5. This figure represents diagrammatically a cross section through the index and dactyl of a normal claw from the left side of a lobster, viewed from the position of the distal end of the appendage. The posterior side of the claw, which is more darkly pigmented in the living lobster, is here shaded, while the ventral side is left clear. The primary morphological relations, as they would obtain if the appendage were extended laterally in a horizontal

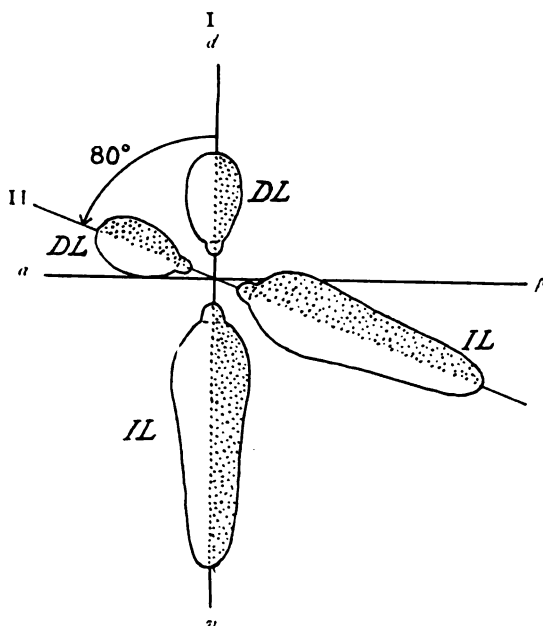


FIG. 5. Diagram to illustrate effects of torsion in a normal left chela. I., primary morphological position; II., position as a result of torsion.

plane and there were no torsion, are shown in the position marked I, where the plane passing through the index (IL) and dactyl (DL) is vertical. Due to torsion, however, the claw is rotated anteriorly¹ nearly 90° (probably ordinarily 75°–80°; for convenience, we may call it 80°), so that its plane approximates the horizontal, as shown in position II. The dactyl thus lies anteriorly, while the posterior surface (shaded) is uppermost. By a bending of the leg now, however, the claws may be brought

¹ By "rotated anteriorly" it is meant that the *dorsal* side has rotated over in the *anterior* direction.

around into the position in which they are normally carried, that is, ahead of the lobster and pointing forward or even inward. As a result the dactyl comes to lie on the side toward the median plane, or even directed posteriorly. The primary claw in Figs. 1 and 2 has nearly the normal position, but as will be pointed out below, the torsion and bending are less than is usual. Emmel (1907, p. 140) has emphasized the importance of taking into account the normal torsion in considering the relations of supernumerary parts in abnormal chelipeds.

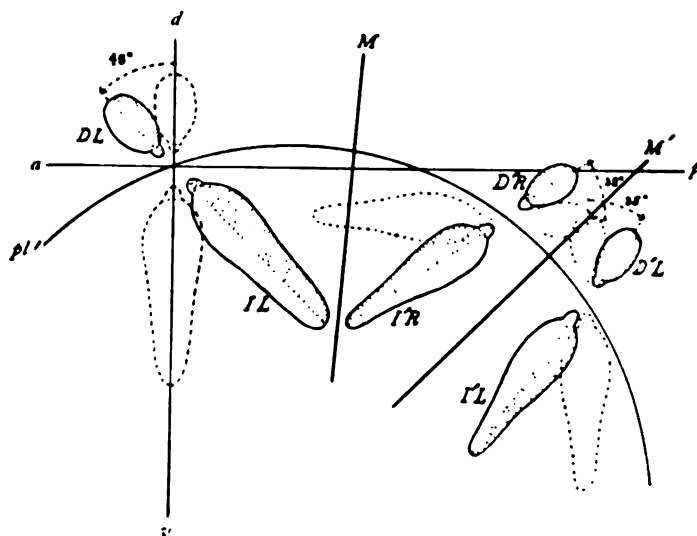


FIG. 6. Diagram showing the spacial relations and secondary symmetry in the abnormal cheliped (full outlines). The dotted outlines show the theoretical relations these parts should have in accordance with the "rules of secondary symmetry," leaving torsion out of account.

In Fig. 6, by a similar diagram, are represented the conditions, as regards rotation, of the three claws of the abnormal specimen. As before, the appendage is considered as being extended straight out laterally from the body. It will be noted that as a result of the abnormal outgrowth the primary claw (*IL*, *DL*) has rotated only about 45° from the vertical plane instead of nearly 90° as in the normal (Fig. 5). The amount of torsion in the abnormal chela will be discussed below with the consideration of the relations of right and left. It may be said here, that as

in the case of the claw, the torsion of the more proximal parts of the limb appears also to have been hindered by the abnormality.

Secondary Symmetry: Dextro-sinestral Relations.—Like most abnormal crustacean appendages with extra processes, the present case falls into the category "in which the extra limb or extra parts of a limb are themselves morphologically double." Furthermore, in accordance with the rules of secondary symmetry laid down by Bateson (1894, p. 479), the normal appendage and the extra parts lie in the same plane¹ and "the nearer of the two extra appendages is in structure and position formed as the image of the normal appendage in a plane mirror [*M*, Fig. 6] placed between the normal appendage and the nearer one, at right angles to the plane of the three axes; and the remoter appendage is the image of the nearer in a plane mirror [*M'*, Fig. 6] similarly placed between the two extra appendages." Thus the two extra claws are morphologically a pair and belong to opposite sides of the body, the inner or proximal one, according to the above rule, being a morphological right (the primary claw being a left), and the outer one a left. This is evident in the diagram (Fig. 6), where it will be noticed by the shading that the relations of the posterior (shaded) and anterior (not shaded) halves are reversed in the middle claw. In one respect, however, the middle claw is not a right; for the normal right claw of this lobster was in all probability of the "nipper" type, the left being a "crusher." As a matter of fact the teeth on both the indices and dactyls of all three of the claws of the abnormal appendage (*IL*, *DL*; *I'R*, *D'R*; *I'L*, *D'L*) are of the "crusher" type. Thus it is evident that while the rules of secondary symmetry hold good for the *spatial relations* they do not apply to the *character* of the parts of the appendage when these normally differ on the two sides of the animal.²

Bateson (1894, p. 479, *et seq.*) has shown that a definite relation obtains between the position which the extra parts assume to each other and to the primary appendage, according to the side of the appendage from which they arise. The theoretical positions he has illustrated plainly by means of a diagram (*loc.*

¹ This plane (Fig. 6, *pl'*) is bent in the present case, owing to torsion. See later.

² Emmel (1907, p. 110) found the same thing to be true in his "specimen No. 5."

cit., Fig. 154) of the leg of an insect, and most of the cases fit in with these expectations very well.

Adapting to the lobster Bateson's diagram for the theoretical positions of the extra parts when they arise from the ventro-posterior (v.p.) side of the normal appendage, as in the present case, we have the relations as shown by the complete outlines

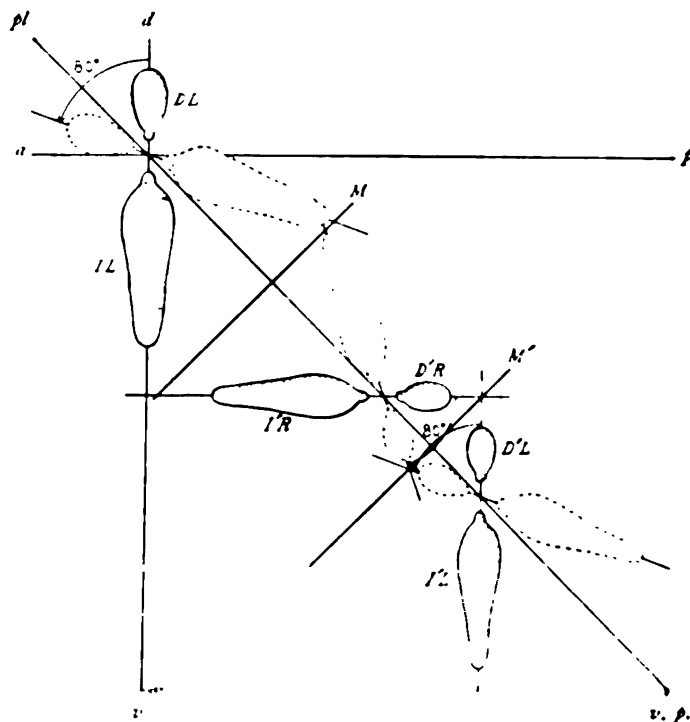


FIG. 7. Diagram illustrating (full outlines) Bateson's "rules of secondary symmetry" as applied to a left lobster chela with double extra claws arising from the ventro-posterior side. The dotted outlines show the positions these parts would assume if each claw were rotated anteriorly, independent of the others, through an arc of 80°.

in Fig. 7, no allowance being made for torsion. But we have seen (p. 259) that the normal lobster claw becomes rotated anteriorly through an arc of approximately 80°. If we now rotate independently toward its anterior side each of the diagrammatic claws in Fig. 7 through an arc of 80°, we arrive at the posi-

tions indicated by the dotted outlines in the same figure. Referring now to Fig. 6, which represents (by the unbroken outlines) the actual positions of the claws, we find that whereas the primary claw has rotated in the expected direction—though only about 45° instead of 80° —the secondary claws appear each to have rotated towards their morphological *dorsal* sides about 35° from the planes prescribed in the theoretical diagram (Fig. 7, full outlines). These relations are perhaps to be accounted for as follows:

While there is some torsion in the base of a normal cheliped, this is compensated for by the rotation of the appendage forward, as a result of which the axis of articulation at the base of the propodite, which is morphologically dorso-ventral, is essentially vertical, as it is in the succeeding pereopods. Hence the position of the great claw is for the most part due to a *torsion in the propodite itself*. In our abnormal specimen, the axis of articulation of the propodite to the carpopodite lies also, as we have seen, practically in a vertical plane; but, apparently as a result of the mechanical hindrance of the secondary growth from its base, the primary propodite has been able to turn only 45° instead of 80° from that plane.

The positions assumed by the secondary claws are a little more difficult of explanation. Since, however, they have a tendency to rotate in opposite directions, the plane (M') midway between them should remain constant in position whatever the amount of torsion, as shown in Figs. 6 and 7, and we may first consider the relation of this plane to that of the primary claw. By reference to Fig. 7 we see that this plane, in the case of a double extra process arising from the postero-ventral surface of an appendage, should make an angle of 45° with the dorso-ventral plane (*d.-v.*). A glance at Fig. 6 will now show that the plane M' there makes the same angle (45°) with the primary dorso-ventral plane of the abnormal specimen. As a result, however, of the torsion distad of the point of divergence, the three claws no longer lie in the same plane ($pl.$, Fig. 7) which passes through the point of origin of the extra parts, but a line connecting their centers has to curve as shown by pl' in Fig. 6.

We have already noted that the planes of the secondary

claws lack 35° of being turned as far as called for by the theoretical positions (solid outlines, Fig. 7), to say nothing of the additional 80° which would be necessary for them to assume the positions as a result of normal torsion (dotted outlines, Fig. 7). As a matter of fact, this torsion should *not* be expected in the secondary claws, since they divide from each other at a considerable distance from the base, and the torsion of the propodite takes place entirely, or practically so, at its base. There would be no torsion in the basal part of the extra outgrowth, since in that region which is potentially double, the tendencies to rotation in opposite directions would neutralize each other. The divergence of the primary claw being further proximal, that has been able to undergo some rotation (45°) as already described. Now as to the failure of the planes of the secondary claws to lie at right angles to each other, as they should, according to Fig. 7, if torsion is eliminated. This is probably to be accounted for upon mechanical grounds, the union of the parts only a short distance proximad interfering with and restricting the divergence of these planes. If the double outgrowth had taken its origin upon a more basal portion of the appendage, it is safe to assume that the planes of the distal claws would have attained more nearly the theoretical positions.¹

The relations of secondary symmetry in the more proximal parts of the appendage appear to be amenable to the same rules; but owing to the way in which the various parts are "compounded" (to use Bateson's term) before they actually divide, this exposition becomes extremely complicated and would serve no useful purpose at this time, since the discussion given above as to the relations of the claws has served to illustrate the factors involved. This compounding accounts for the absence of large spines along the dorsal side of the extra propodite, which has already been mentioned.

¹ In Emmel's interesting "specimen No. 5" the extra chelæ arose from the meropodite, and he here found (the union being further proximad) that each of the claws had undergone a full rotation, each in its normal direction of torsion. According to his description (Emmel, 1907, p. 141) the primary or normal claw had rotated 90° , but *each of the extra claws had rotated 180°* ! Why this should have been seems difficult to understand and Emmel offers no explanation. Why, with the direction of torsion opposed in contiguous claws, its amount should be limited to less than 90° , as in the specimen here described, is obvious; but why in any case it should be *greater* than 90° seems inexplicable.

CAUSE OF THE ABNORMALITY.

While believing that we must ultimately look to experiment for the elucidation of the causes of abnormalities of this sort, there are nevertheless certain considerations in the present instance which are of interest. On the theory that they may be the result of injuries, marks indicating such injury have often been sought. The scar of an evident injury is very noticeable on the carpopodite of the present specimen (Fig. 3, s.); but it is equally evident that this injury cannot have been the cause of the abnormality, since, as we have seen, the effect of the doubling is obvious as far proximad as the ischiopodite. It seems more probable that after some antecedent autotomy of the appendage the regenerating bud was in some way injured or disturbed so as to produce the abnormality. A definite scar for such an injury could not be expected in the fully developed appendage. Not only would it be of the greatest importance to know whether this abnormality would be reproduced if the leg could have been amputated autotomously and allowed to regenerate (cf. Emmel, 1907, p. 111), but it would also be interesting to know whether a lobster could moult normally with an appendage of this sort. On account of the extra amount of material involved, which would have to be drawn through the narrow opening at the base of the leg, it would certainly be a much more precarious ordeal than usual.

SUMMARY.

In this paper is described the abnormal cheliped of a lobster (*Homarus americanus*), the abnormality consisting of a double extra claw. While the extra part actually separates from the normal propodus, it is shown that the effects of the doubling may be traced to the base of the leg. It is further shown that the conditions in this appendage illustrate almost diagrammatically the "rules of secondary symmetry" formulated by Bateson, if allowance be made for the effects of the torsion which occurs in the normal lobster cheliped and the mechanical conditions which may modify such torsion.

ADDENDUM.

Since it is of advantage to place on record where they may be found the cases of abnormal crustacean appendages, it may be well to call attention here to two recent records which might naturally be overlooked. They both occur on crabs, and both belong to the class of "extra processes arising from the normal dactyl," being directly comparable to the chelæ figured by Emmel (1907, pl. 1, Fig. 1), Faxon (1881, pl. 1, Figs. 1, 2, 3, 4, 5, 7, 8), Herrick (1895, pl. 47, Fig. 191), and Bateson (1894, Figs. 184, 185).

The first case is figured incidentally by Verrill (1908, p. 396, Fig. 37) in his report on the "Decapod Crustacea of Bermuda."

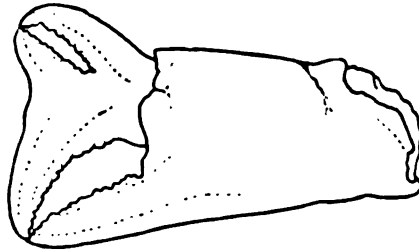


FIG. 8. Deformed claw of an undetermined cancrivore crab. (After Verrill.)

Fig. 8 represents the condition of this specimen, which is described as the "deformed claw of an undetermined cancrivore crab."

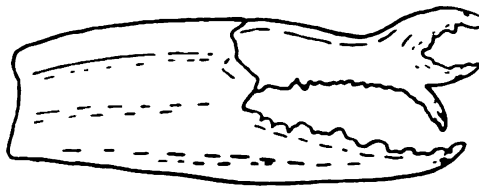


FIG. 9. Abnormal crab claw from Stonington, Conn. (After Leavitt)

The second case (Leavitt, 1909), illustrated in Fig. 9, is of a crab, possibly *Callinectes*, from Stonington, Conn. The author states that there was a brief mention of this claw in *St. Nicholas* for December, 1907. He makes the mistake of considering this a reduplication of the entire claw, except that "in the small pincer the dactyl is not movable at the base, as it is in the larger

one." Further, he calls it a case of "homœosis" and gives a general consideration of it in relation to Weismannism, etc.

LITERATURE.

Bateson, W.

'94 Materials for the study of variation, treated with especial regard to discontinuity in the origin of species. London, xvi + 598 pp.

Emmel, V. E.

'07 Regenerated and abnormal appendages in the lobster. 37th Ann. Rept. Commissioner of Inland Fisheries, Rhode Island, pp. 99-152, pl. 1-9.

Faxon, W.

'81 On some crustacean deformities. Bull. Mus. Comp. Zool., vol. 8, no. 13, pp. 257-274. pls. 1, 2.

Herrick, F. H.

'95 The American lobster: A study of its habits and development. Bull. U. S. Fish Comm., 1895, pp. 1-252, pl. A-J, 1-54.

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'09 An interesting crab's claw. The Guide to Nature, vol. 3, no. 3, pp. 89-91.

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EXPLANATION OF ABBREVIATIONS USED IN FIGURES.

a., anterior.

a'., secondary point of articulation of ischiopodite to meropodite.

a.p., anterior articular process of meropodite.

C., carpopodite.

C + [C'(R + L)]., carpopodite of abnormal specimen, compounded of the primary and secondary parts.

d., dorsal.

d.a., primary (normal) dorsal articulation of ischiopodite to meropodite.

DL, left primary or normal dactyl.

D'L, left extra dactyl.

d.p., primary (normal) dorsal articular process of carpopodite.

d.p'., extra (secondary) dorsal articular process of carpopodite.

D'R., right extra dactyl.

I, index.

IL, left primary or normal index.

I'L, left extra index.

I'R, right extra index.

Is, ischiopodite.

M, line representing position of a plane mirror placed midway between the primary claw and the nearer secondary claw, and normal to the plane in which they lie.

M', line representing position of a plane mirror placed midway between the extra claws and normal to the plane in which they lie.

Mer., meropodite.

p., posterior.

p'., process representing an incipient secondary articular axis between meropodite and carpopodite.

pl., plane passing through the point of origin of the secondary growth from the primary claw and through both the extra claws.

p'., curved line representing the bending of the plane *pl*. as a result of torsion and the mechanical conditions.

pp., primary (normal) posterior articular process of meropodite.

Pr., propodite.

PrL, left primary or normal propodite.

Pr' (*R+L*), extra double propodite.

r., ridge at base of ischiopodite, possibly representing a secondary incipient point for articulation with basal podomeres.

s., scar on base of abnormal carpopodite.

sp., large spine on meropodite.

SpDL, spine at base of primary or normal dactyl.

SpD'L, spine belonging to base of left extra dactyl.

SpD'R, spine belonging to base of right extra dactyl.

v., ventral.

v.a., primary (normal) ventral articulation of ischiopodite to meropodite.

v.p., primary (normal) ventral articular process of carpopodite.

v.p'., extra (secondary) ventral articular process of carpopodite.

v.-p., ventro-posterior, the side of the primary claw from which the extra claws arise.

x., smooth spot where the secondary dorsal articular process (*d.p'*.) of the carpopodite has rubbed against the base of the propodite.

I., position before torsion.

II., position as a result of torsion.

METHODS OF ARTIFICIAL PARTHENOGENESIS.

E. NEWTON HARVEY.

Papers on the subject of artificial parthenogenesis have been fairly abundant during the past ten years, yet somewhat scattered, appearing in zoological, physiological and chemical journals. It therefore seemed desirable to list the important contributions, giving briefly, but in detail, the various methods of exciting eggs to develop, together with results and references, in the hope that it might prove of value to future workers.

Such a list follows. Only annelids, echinoderms, molluscs and vertebrates have been included. Under each class the observers are arranged in alphabetical order; their researches in chronological order.

As the most important general and theoretical work on artificial parthenogenesis may be mentioned the recent book of Loeb, "Die Chemische Entwicklungserregung des tierischen Eies," Berlin, 1909, in which the various phases of this most interesting subject are discussed in considerable detail.

The following abbreviations have been used:—

<i>s. w.</i> sea water.	<i>dist. w.</i> distilled water.
<i>m.</i> molecular solution.	<i>P. B.</i> polar bodies.
<i>n.</i> normal solution.	<i>esp.</i> especially.
<i>min.</i> minute.	<i>P. D.</i> potential difference.
<i>hr.</i> hour.	

ANNELIDS.

Egg of	Preliminary Treatment.	After Treatment and Remarks.	Per cent.	Observer.	Reference.
<i>Ophelia</i> .	Hypertonic sea water (20 c.c. 2.5 m. KCl + 80 c.c. a.w. for 2 hrs. at 18°).	To a.w. Swimming larvae.	60	Bullot, G.	<i>Arch. Entom.</i> , XVIII., p. 161, 1904 and <i>U. Calif. Pub. Physiol.</i> , I., p. 165, 1904.
<i>Amphitrite</i> .	Hypertonic sea water (98 c.c. a.w. + 2 c.c. 2.5 m. $\text{Ca}(\text{NO}_3)_2$ for 30 min.).	To a.w. Trochophores.		Fischer, M. H., at Woods Hole.	<i>Am. J. Physiol.</i> , 7, p. 301, 1902.
"	Calcium ions (98 c.c. a.w. + 2 c.c. m. Ca salt).	Permanently. Mg. Li and Na cause no development.		Fischer, M. H., at Woods Hole.	<i>Am. J. Physiol.</i> , 7, p. 301, 1902.
"	Mechanical agitation.			Fischer, M. H., at Woods Hole.	<i>Am. J. Physiol.</i> , 7, p. 301, 1902.
<i>Nereis</i> .	Hypertonic sea water by NaCl. KCl or cane sugar (17.5 c.c. 2.5 m. KCl + 82.5 c.c. a.w. for 1 hr.).	To a.w. Swimming larvae.		Fischer, M. H., at Woods Hole.	<i>Id.</i> , 9, p. 100, 1903.
<i>Thalassidroma medusa</i> .	Acids (mineral, fatty, dibasic organic and CO_2). To a.w. Trochophores. 17 c.c. m/10 HNO_3 + 83 c.c. a.w. for 5 m. 15 c.c. m/10 HCl + 85 c.c. a.w. for 5 m. 10 c.c. m/20 H_2SO_4 + 90 c.c. a.w. for 8 m. 12 c.c. m/20 $(\text{COOH})_2$ + 88 c.c. a.w. for 8 m. 15 c.c. m/10 CH_3COOH + 85 c.c. a.w. for 5 m. CO_2 from generator to a.w. for 10 m. and eggs immersed 1 hr.		6 to 60	Lefevre, G., at Beaufort, N. C.	<i>Journ. Exp. Zool.</i> , IV., p. 90, 1907.
<i>Chelodermis</i> .	KCl sea water (10-20 c.c. 2.5 m KCl + 90 c.c. a.w. for 1 hr.)	Confirms Loeb.		Lillie, F. R.	<i>Arch. Entom.</i> , XIV., p. 477, 1902.
<i>Chelodermis</i> , <i>Phascolosoma</i> , <i>Podarke</i> .	Hypertonic sea water (15-20 c.c. 2.5 m NaCl + 85 c.c. a.w. for 1 hr.).	To a.w. "swimming larvae."		Loeb, J., at Woods Hole.	<i>Am. J. Physiol.</i> , IV., p. 423, 1901, and <i>Science</i> , XII., p. 170, 1900.
<i>Chelodermis</i> .	Potassium ions (10 c.c. 2.5 m KCl + 90 c.c. dist. w. for 7 m.			Loeb, J., at Woods Hole.	<i>Am. J. Physiol.</i> , IV., p. 423, 1901, and <i>Science</i> , XII., p. 170, 1900.

ANNELIDS.—Continued.

Egg of	Preliminary Treatment.	After Treatment and Remarks.	Per cent.	Observer.	Reference.
<i>Chaetopterus</i> .	Acids (100 c.c. s.w.+1-2 c.c. HCl and left permanently).	Segmentations.	Small	Loeb, J., at Woods Hole.	<i>Amer. J. Physiol.</i> , IV., p. 423, 1901, and <i>Science</i> , XII., p. 170, 1900.
<i>Polynoë</i> , <i>Sipunculus</i> .	Alkalies (50 c.c. s.w.+1.5 c.c. π /10 NaOH) permanently or 5-6 hrs.	To s.w. Larvæ.	30 30	Loeb, J.	<i>Pfugger's Archiv.</i> , 118, p. 572, 1907, and <i>U. Calif. Pub. Phys.</i> , 3, p. 71, 1907.
<i>Polynoë</i> .	Alkali (dilute) for 4 hrs.	50 c.c. 5/8 m. Vant' Hoff's sol.+10 c.c. 2.5 m. NaCl for 2 hrs.			<i>U. Calif. Pub. Phys.</i> , 3, p. 71, 1907.
"	Saponin and solanine (15 drops weak sol. in 4 c.c. s.w. for 1-1.5 min.)	To s.w. Larvæ.	Most	Loeb, J.	<i>Arch. f. d. ges. Physiol.</i> , 122, p. 448, 1908.
<i>Amphitrite</i> .	Hypertonic sea water. Calcium Agitation.	Confirms Fischer.		Scott, J. W., at Woods Hole.	<i>J. Exp. Zool.</i> , 3, p. 62, 1906.
<i>Podarke</i> .	Hypertonic sea water (20 c.c. 2.5 KCl+80 c.c. s.w. for 1 hr.).	To s.w. swimming larvæ.		Treadwell, A. L., at Woods Hole.	<i>BIOL. BULL.</i> , 3, p. 235, 1902.

HOLOTHURIANS.

<i>Holothuria tubulosa</i> .	Acids (3 c.c. π /10 HCl+100 c.c. s.w. for 5 min.).	To s.w. Segmentations but no larvæ.		Lyon, E.P. at Naples.	<i>Am. J. Physiol.</i> , IX., p. 308, 1903.
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MOLLUSCS.

Egg of	Preliminary Treatment	After Treatment and Remarks.	Per cent.	Observer.	Reference.
<i>Macra</i> .	Hypertonic sea water (5-15 c.c. 2.5 π KCl, NaCl, CaCl ₂ to 85-95 c.c. s.w. for .5-2 hrs.) Concentrated (by evap.) s.w.	To s.w. Segmentations.		Kostanecki, K., at Naples.	<i>Arch. f. micros. anal.</i> , 64, p. 1, 1904, also <i>id.</i> , 72, p. 327, 1908.
<i>Lotia gigantea</i> .	Hypertonic sea water (15-20 c.c. 2.5 π KCl to 100 c.c. s.w. for 2 hrs.).	To s.w. Swimming larvæ.		Loeb, J., at Pacific Grove.	<i>U. Calif. Pub. Phys.</i> , 1, 1903, p. 7.
<i>Arcea</i> sp.	10 c.c. 2.5 π KCl to 100 s.w. 2 hrs.	To s.w. Swimming larvæ.		Loeb, J.	<i>Id.</i>

MOLLUSCS.—Continued.

Size of	Preliminary Treatment.	After Treatment and Remarks.	Per Cent.	Observer.	Reference.
<i>Lottia gigantea</i> .	Hypertonic alkaline solution (50 c.c. 5/8 M Van't Hoff + 10 c.c. 2.5 M NaCl + 0.5 c.c. N/10 NaOH for 2 hrs.	For artificial maturation methods see To a.w. Swimming larvae.	Almost all.	Loeb, J. M. Wolfsohn, J.	<i>Id.</i> , III., p. 1, 1905. BIOL. BULL., 13, p. 344, 1907. <i>Arch. f. d. ges. Physiol.</i> , 118, p. 575, 1907.
STAR-FISH.					
<i>Asterias glacialis</i> .	Hypertonic sea water (by K. Na, Mg. .73 M.) To a.w. Blastulae for 1 hr.			Delage, Y.	<i>Arch. d. Z. Exp. et Gen.</i> , 3d, 9, p. 307, 1901.
"	Heat (a.w. at 28°-35° allowed to cool by radiation or by cold).	To Blastulae.	25	"	<i>Id.</i> , p. 307.
"	Acid (HCl 0.1-0.2 g. per L.) to a.w.	"	50	"	<i>Id.</i> , p. 309.
"	Manganese sea water. a.w. .260 .506 m. } .506 m. MnCl ₂ .246	Heat + KCl a.w. to a.w. Heat + HCl a.w. to a.w. To a.w. segmentations. MnCl ₂ a.w. + HCl to a.w.	40 100 5	"	<i>Id.</i> , p. 313.
"	.52 m. MnCl ₂	To a.w.	45	"	<i>Id.</i> , 4th, 3, Notes et Revue, p. clxvi; also C. R. Ac. Sc. Paris, 140, p. 1369, 1905.
"	CO ₂ Charged a.w. for 1 hr. (between break-down of germinal vesicle and appearance of 9 pronuc.).	To a.w. "Auricularia."	100	"	<i>Id.</i> , 3d, 10, p. 213, 1902, and C. R. Ac. Sc. Paris, 135, p. 605, 15, 570, <i>Id.</i> , 137, p. 473.
"	Mechanical agitation.	Segmentation and a few blastulae.	5-30	"	
"	Sodium phosphate sea water. ¹ NaH ₂ PO ₄ .270 } .625 m a.w. .355	Numerous larvae.	3	"	<i>Arch. d. exp. Zool. et Gen.</i> , 4th, 3, Notes et Revue, p. clxviii, 1905.

¹ Efficiency probably due to presence of H ions.

STAR-FISH.—Continued.

Egg of	Preliminary treatment	After Treatment and Remarks	Per cent.	Observer.	Reference.
<i>Asterias forbesii</i> .	Cold (s.w. at 4°-7° C. for 1-9 hrs., after maturation).	Bipinnaria larvæ.	20	Greeley, A.	<i>Am. Journ. Physiol.</i> , VI., p. 296, 1902.
<i>Asterias forbesii</i>	Heat (s.w. at 35°-70 min.; 36°-45 min.; 37°-30 min. 38°-20 min.; after dissolution of germinal vesicle and before separation of 1st. P.B.).	To s.w. Bipinnaria larvæ.	High.	Lillie, R. S.	<i>J. Exp. Zool.</i> , V., p. 375, 1908.
"	m/2000 KCW in s.w. for 50 min., then heat (as above).	To s.w. Bipinnaria larvæ.	100	"	<i>J. Exp. Zool.</i> , V., p. 375, 1908.
<i>Asterina</i> .	m/2000 KCN in s.w. for 50 min., then heat to m/2000 KCN in s.w. for 5-10 min.	To s.w. Bipinnaria larvæ.	100	"	<i>J. Exp. Zool.</i> , V., p. 375, 1908.
	Acids (5 c.c. m/10 fatty acid + 50 c.c. s.w. for 1-2 m.).	To s.w. Blastulæ.	10	Loeb, J.	<i>U. Calif. Pub. Physiol.</i> , II., p. 147, 1905.
	Fat solvents (amylen, benzol, sat. sol. in s.w.).	Membranes formed.		"	<i>Id.</i> , p. 149.
<i>Asterias</i> .	Acids (3-5 c.c. m/10 inorganic acid esp. HCl to 100 c.c. s.w. for 3-20 min.).	To s.w. swimming larvæ.	20	Loeb, J., Fischer, M. and Neilson, H.	<i>Arch. f. d. ges. Physiol.</i> , 87, p. 1, 1901.
		For substances causing maturation see		Loeb, J.,	<i>Biol. Bull.</i> , III., p. 295, 1902.
<i>Asterias forbesii</i> .	Mechanical agitation (after maturation; 3 hrs. after shedding optimum time).	Bipinnaria larvæ and gastrulæ.	50	Matthews, A. P., at Woods Hole.	<i>Am. J. Physiol.</i> , VI., p. 142, 1902.
<i>Asterias</i> .	Distilled water (1 min.).	To s.w. Larvæ.		Schücking, A., at Naples.	<i>Arch. f. d. ges. Physiol.</i> , 97, p. 85, 1903.
"	Electric current (constant, from 2 chromic acid elements). 2 min.	Segmentation.		Schücking, A., at Naples.	<i>Id.</i>
"	Acids (acetic and citric) 2 drops conc. CH ₃ COOH to 200 c.c. s.w. for 40 min.).	To s.w. larvæ.		Schücking, A. at Naples.	<i>Id.</i>

STAR-FISH.—Continued.

Egg of	Preliminary treatment	After Treatment and Remarks.	Per cent.	Observer.	Reference.
<i>Asterias</i> .	NaHCO_3 (1.5 per cent. for 1.25 hrs.). Heat (a.w. at 34°C).	To a.w. Bipinnaria and blastulae.		Schlücking, A., at Naples.	<i>Arch. f. d. ges. Physiol.</i> , 97, p. 85, 1903.
SEA-URCHINS.					
<i>Strongylocentrotus lividus</i> .	Hypertonic solutions (by KCl , NaCl , MgCl_2): a.w. .317 ¹ } .817 m for 1.5 hrs. KCl .500 }	To a.w. Segmentations and blastulae.	80 75	Delage, Y.	<i>Arch. d. zool. Exp. et General.</i> , 3d, 9, 1901, p. 296. <i>Id.</i> , p. 311.
<i>Strongylocentrotus lividus</i> .	Manganese sea water. a.w. .156 } .648 m. MnCl_2 .492 }	To a.w. Segmentations.	15	"	"
<i>Strongylocentrotus lividus</i> .	CO_2 sat. a.w. at $28^\circ\text{--}30^\circ\text{C}$. (allowed to cool by radiation) for 1 hr.	To a.w. Larvæ.	60	"	<i>Id.</i> , 4th, 2, pp. 43-46, 1904.
<i>Strongylocentrotus lividus</i> .	Nickel and Cobalt sea water (100 c.c. hyper- tonic a.w. .95 m + 1-1.5 c.c. m NiCl_2).	To a.w. CoCl_2 less effective. More effective when Ni applied after hypertonic sol.		"	C. R. Ac. Sc. Paris, 143, p. 863, 1906.
<i>Strongylocentrotus lividus</i> .	Na hyposulphite sea waters (100 c.c. hyper- tonic a.w. .95 m + 5 to drops m Na hyposulphite). Acid and alkali strong hypertonic sol. (11.5 Weak hypertonic sol. High. c.c. 2.5 m NaCl + 2.5 c.c. a.w. + 50 c.c. dist. w.) (37.5 c.c. 2.5 NaCl + + 17 drops m/10 HCl for 5-6 min.) 2.5 c.c. a.w. + 60 c.c. dist. W.) + 17 drops NH_4OH for 1 hr. 20 m.; to a.w.			"	C. R. Ac. Sc. Paris, 143, p. 863, 1906. <i>Arch. d. zool. exp. et gen.</i> , 4th, 7, 1907, p. 445, also C. R. Ac. Sc. Paris, 145, p. 218, 1907.
	HNO_3 , H_2SO_4 , $(\text{COOH})_2$, CH_3COOH , HCOOH NaOH ; $\text{Ca}(\text{OH})_2$, Na_2 favorable but not H_3PO_4 , citric, lactic, butyric, tartaric, valericanic or boric	CO_2 favorable but not KOH . Treatment cannot be reversed Larvæ obtained.		"	

¹ Gram molecules of the salt in a liter of water. 5/8 m. .625 m. Efficiency probably due to increase of OH ions.

SEA-URCHINS.—Continued.

Egg of	Preliminary treatment	After Treatment and Remarks.	Per cent.	Observer.	Remarks.
<i>Strongylocentrotus lividus</i> .	Tannin and ammonia—50 c.c. sol. (hypo-iso- and hypertonic sols. of various salts, salt mixtures and sugar)+28 drops $\pi/10$ tannin ($C_{12}H_{10}O_9=322$) for 5-6 min. Orcinol, resorcinol, phloroglucine, pyrocatechinol, phenol, pyrogallol, oxyhydrochinone, picric, salicylic, vanillic, gallic and protocatechuic acids may be substituted for tannin. Electric charges (P. D. 15 volts+elec. for 30 m.,—elec. for 1.25 hrs. in 70 c.c. m. cane sugar+30 c.c. s.w. Cold (s.w. at 0°-5° C. for 1-6 hrs.).	Then 30 drops $\pi/10$ NH_4OH added. After 1 hr. to s.w. The most favorable medium is 70 c.c. in cane sugar+30 c.c. s.w. Larvæ obtained.	High.	Delage, Y.	<i>Arch. d. z. exp. et gen.</i> , 4th, 7, 1907, p. 445, and <i>C. R. Ac. Sc. Paris</i> , Nov. 4, 1907.
<i>Strongylocentrotus lividus</i> .		To s.w. segmentations and a few larvæ.	Many.	"	<i>Arch. d. z. exp. et gen.</i> , 4th, 9, Notes et Revue, XXX., 1908.
<i>Arbacia punctulata</i> .		Irregular segmentation.	Small.	Greeley, A. W., at Woods Hole, also Morgan, T. H. Herbst, C.	<i>Am. J. Physiol.</i> , VI., p. 296, 1902.
Sea urchins		Membranes formed.		"	<i>Arch. f. Entw.</i> , X., p. 489, 1900.
<i>Strongylocentrotus lividus</i> .	Fat solvents (toluol, benzol, xylol, creasote and oil of cloves). Silver and copper salts (metallic Ag and Cu in s.w. also AgCl). No results with Fe, Ni, Pb, Pt or Au.	" "		"	<i>Biol. Centralb.</i> , 13, p. 14, 1893.
<i>Sphaerechinus</i> .	Acid (50 cc. s.w.+3 c.c. $\pi/10$ CH_3COOH for 4-6 min.). $CHCl_3$ sat. sea water.	To s.w. Plutei.		"	<i>Mittheil. aus d. z. Stat. zu Neap.</i> , 16, p. 445, 1904.
<i>Echinus microstomus</i> .		Membranes formed.		"	<i>Arch. Entw.</i> , 22, p. 473, 1906.
<i>Echinus</i> .	Strychnin (0.1 per cent. strychnin sulphate for .5-3 hrs.).	To s.w. Division.		Hertwig, O. and R.	"Untersuchungen z. Morphologie u. Physiol. der Zena," H. 5, Jena, 1887.
				Hertwig.	"Festschrift für gegenbauer," 2, p. 21, 1896.

* Loeb has shown that these solutions are all hyperosmotic (*C. R. Ac. Sc. Paris*, 146, p. 246, 1908). Isotonic sols. are not necessarily isosmotic (Loeb, *Biochem. Zeit.*, II, p. 144, 1908).

SEA-URCHINS.—Continued.

Egg of	Preliminary treatment.	After Treatment and Remarks.	Per cent.	Observer.	Reference.
<i>Arbacia</i> .	Concentrated sea water (by evap.; 500 c.c. s.w. evap. to 250-375 c.c.; for 2 hrs.).	Segmentation and gastrule.	90 40	Hunter, S. J., at Woods Hole.	<i>Am. J. Physiol.</i> , VI., p. 177, 1902.
<i>Strongylocentrotus purpuratus</i> and <i>franciscanus</i> .	Sperm extract (killed by 70-100° and filtered; To a.w. at 8°-10° C. or to hypertonic a.w. (8 c.c. 2.5 NaCl+50 c.c. a.w.) for 35-50 min.		Few.	Kupelwieser, H.	<i>Biol. Centralb.</i> , 26, p. 744, 1906.
<i>Arbacia</i> , <i>Strongylocentrotus</i> .	Hypertonic sea water (50 c.c. 20/8 M MgCl ₂ , To a.w. Plutei. + 5 c.c. a.w. for 2 hrs.), also with NaCl, sugar, urea (10 c.c. NaCl or KCl 2.5 M + 90 c.c. a.w. for 2 hrs.).		90	Loeb, J., at Woods Hole and Pacific Grove.	<i>at Am. Journ. Physiol.</i> , III., p. 135, 1899; IV., p. 178, 1900; III., p. 434, 1900.
<i>Strongylocentrotus purpuratus</i> and <i>franciscanus</i> .	Acids (monobasic fatty). 0.6 c.c. m. ethyl acetate ¹ to 50 c.c. a.w.	Hypertonic a.w. (7-8 c.c. 2.5 M KCl+50 c.c. a.w. for 25-50 m.) to a.w. Larve obtained.	90-100	Loeb, J.	<i>U. Calif. Pub. Physiol.</i> , II., p. 83, 1905.
	3 c.c. M/10 fatty acid to 50 c.c. a.w. for 2-3 m. Hypertonic a.w. 25-50 c.c. M/10 HNO ₃ to 50 for 2.5 min.		90-100	"	<i>Id.</i> , p. 113.
	Hypertonic a.w. (7.8 c.c. 2.5 M KCl+50 c.c. a.w. for 1.5 2 hrs.).	3 c.c. M/10 CH ₃ COOH + 50 c.c. a.w. for 2-3 min. to a.w.	Few. 90-100	"	<i>Id.</i> , p. 89.
<i>Strongylocentrotus</i> .	Fat solvents (eal. sola. of toluol, amylene, etc., in a.w.) phenol (6 c.c. M/2 phenol to 50 c.c. a.w.).	After membrane formation, hypertonic treatment to a.w. Larve.	Small.	"	<i>Id.</i> , III., p. 74, 1907.
"	Alkalies (50 c.c. 5/8 M Vant' Hoff's sol. + 0.5 1.0 c.c. M/10 NaOH for 2 hrs.). Hypertonic Vant' Hoff's sol. (50 c.c. sol. + 8 c.c. 2.5 NaCl for 2 hrs.).	Hypertonic a.w. for 30 50 m. to a.w. plutei. 50 c.c. 5/8 sol. or a.w. + 1.5 c.c. NaOH for 1.5 -2hrs.	Majority.	"	<i>Arch. f. d. ges. Physiol.</i> , 118, p. 30, 1907, and 118, p. 572, 1907.

¹Free CH₃COOH present.

SEA-URCHIN.—Continued.

Egg of	Preliminary treatment.	After Treatment and Remarks.	Per cent.	Observer.	Reference.
<i>Strongylocentrotus</i> .	Blood sera (of worms, <i>Dendrosoma</i> and <i>Sipunculus</i> diluted 1,000-5,000 times with a.w.).	To a.w. after membrane formation gives 16-32 cell stages. Hypertonic treatment gives plutei.	10-90	Loeb, J.	<i>Arch. f. d. ges. Physiol.</i> , 118, p. 36, 1907, and <i>U. Calif. Pub. Physiol.</i> , 3, p. 57, 1907.
"	Saponin, solanin, digitallin. (A trace in 5 c.c. a.w. for 5-8 m.) Bile salts (Na glycocholate and Na taurocholate). Heat (slow warming to 34°-35°). Blood serum (rabbit—0.7 c.c.+1 c.c. 2.5 NaCl or 4 c.c. a.w.+4 drops serum).	Hypertonic sea water for 35-80 min.; larvae. Hypertonic sea water for 35-80 min.; larvae. Membranes formed. After membranes formed and hypertonic treatment, larvae produced.	10-80	"	<i>Arch. f. d. ges. Physiol.</i> , 122, p. 196, 1908.
"	Blood serum (pig and ox) treated as with rabbit serum.	Sr and Ba increase activity, Mg and Ca decrease activity of serum.	Most.	"	<i>Arch. f. d. ges. Physiol.</i> , 122, p. 196, 1908.
"	Soaps (only if trace free acid present). Membrane formation by any method.	Hypertonic solutions for 58 min. at 15° C. Saccharose 0.06 m., Glucose 1.04 m., NaCl 0.79 m., KCl 0.78 m., LiCl 0.74 m., CaCl ₂ 0.50 m., MgCl ₂ 0.47 m., SrCl ₂ 0.78 m., Urea 1.50 m.,	80 90 90 80 80 85 80	"	<i>Id.</i> , p. 45. <i>Biochem. Zeit.</i> , 11, p. 144, 1908.
Sea urchins of California coast.				"	<i>Id.</i> , p. 45. <i>Biochem. Zeit.</i> , 11, p. 144, 1908.

SEA-URCHINS—Continued.

Egg of	Preliminary treatment.	After Treatment and Remarks.	Per cent.	Observer.	Reference.
Sea-urchins of California coast.	Membrane formation by any method.	Hypertonic sea water for 25-50 min.		Loeb, J.	<i>Id.</i>
Sea-urchins of California coast	"	50 c.c. a.w.+8 c.c. 2.5 KCl. 50 c.c. a.w.+6.5 c.c. 2.5 LiCl. 50 c.c. a.w.+5 c.c. 2.5 CaCl ₂ . 50 c.c. a.w.+4.5 c.c. 2.5 MgCl ₂ . 50 c.c. a.w.+12 c.c. a.w. MgSO ₄ . 50 c.c. a.w.+14.4 c.c. 2.5 glucose. 50 c.c. a.w.+10 c.c. 2.5 saccharose.		"	
Sea-urchins of California coast.	"	Sea water at 2-5° C.		"	Article on parthenogenesis in Oppenheimer's "Handbuch der Biologie des Menschen u. der Tiere, B. II., 1st half, p. 97, Jena, 1909. Also <i>Biochem. Zelt.</i> , I., p. 202, 1906.
Sea-urchins of California coast.	"	Oxygen-free a.w. by current of hydrogen.		"	
Sea-urchins of California coast.	"	KCN sea water (1.5-1 c.c. 0.1 per cent. KCN+50 c.c. a.w. for 5-8 hrs.).		"	
Sea-urchins of California coast.	"	Chloral hydrate a.w. (3 c.c. a/10 chloral hydrate +50 c.c. a.w. for 3-6 hrs.).		"	

SEA-URCHINS.—Continued.

Key of	Preliminary treatment.	After Treatment and Remarks.	Per cent.	Observer.	Reference.
<i>Arbacia pustulata</i> and <i>Strongylocentrotus lividus</i> .	Acids (2-7 c.c. π /10 HCl+100 c.c. s.w. for 5-15 min.) (dilute (1/2-1/6 sat.) CO_2 sea water 2-30 min.)	To s.w. Plutei. To s.w. segmentations and a few larvæ.	10	Lyons, E. P., at Naples.	<i>Am. Journ. Physiol.</i> , IX., p. 308, 1903.
	Hypertonic sea water (10-16 c.c. 2.5 π KCl or NaCl+100 c.c. s.w. for 1-2 hrs.). KCN π /100- π /1,000 for 1-2 days.	To s.w. Plutei.	High.	Lyons, E. P., at Naples.	<i>Am. Journ. Physiol.</i> , IX., p. 308, 1903.
	O-free sea water (by H gas; 30 min. or longer).	To s.w. No larvæ but segmentations. To s.w. Divisions.	High.	Lyons, E. P., at Naples.	<i>Am. Journ. Physiol.</i> , IX., p. 308, 1903.
<i>Arbacia</i> .				Mathews, A. P., at Woods Hole.	<i>Am. J. Physiol.</i> , IV, p. 343, 1900.
"	Alcohol (10 c.c. s.w.+1 c.c. 50 per cent. $\text{C}_2\text{H}_5\text{OH}$ for 10-15 min.). Also saturated ether and CHCl_3 sea water.	To s.w. Divisions.			
"	Pilocarpine, strychnine and quinine. Distilled water (1 min.).	To s.w. Divisions.			
<i>Arbacia pustulata</i> .		To s.w. Larvæ.		Schücking, A.	<i>Arch. f. d. ges. Physiol.</i> , 97, p. 85, 1903.
	Cold (3-4° C. for 3 hrs.). NaHCO_3 (1.5 per cent. for 10 min.).	To s.w. Blastulæ. To s.w. Gastrulæ.		"	<i>Id.</i>
<i>Strongylocentrotus lividus</i> .	Nicotin, hyocyanin, strychnine (1 drop nicotin to 100 c.c. s.w.); 1 part 0.25 per cent. hyocyanin+3 parts s.w.	After 2 hrs. to s.w. Irregular segmentation.		"	<i>Id.</i>
<i>Toxopneustes</i> .	Hypertonic sea water (1 part s.w.+ 1 part 20/8 π MgCl_2 for 1-2 hrs.).	To s.w. Plutei.		Wasallieff, A., at Villefranche.	<i>Biol. Centralbl.</i> , 22, p. 738, 1902.
				Wilson, E. B. at Beau-fort.	<i>Arch. Entwem.</i> , XII., p. 529, 1901.

VERTEBRATES.

Key of	Preliminary treatment.	After Treatment and Remarks.	Per cent.	Observer.	Reference.
<i>Leuciscus nalis</i> .	Hypertonic solutions (10 per cent. cane sugar 3 hrs., 1 per cent. NaCl 3 hrs.)	To water. Irreg. segmentations but furrows later disappear; development questionable.	50	Bataillon, E.	Arch. Entw., XI., p. 169, 1900, and C. R. Ac. Sc. Paris, 134, p. 918, 1902.
<i>Rana esculenta</i> .	Ox serum. 1-2.5 hrs.	To water. Segmentation	90	"	Arch. Entw., 18, p. 17, 1904, and C. R. Ac. Sc. Paris, 137, p. 79, 1903.
<i>Rana fusca</i> .	Heat (water at 35° for 30 min.).	To water. Segmentation		"	
"	Hypertonic soln. (6 per cent. cane sugar, 8-15 hrs.)	To water. Segmentation		"	
"	Heat+6-8 per cent. cane sugar.	Blastula.		"	
<i>Peromyscus leuceri</i> .	Hypertonic soln. 6 per cent. cane sugar.	Segmentation.		"	
Fish.	Diphtheria antitoxin.	Segmentation?		Kuhagin.	Zool. Anz., 1898, p. 653.

PELAGOSPHERA, A LARVAL GEPHYREAN.

HAROLD HEATH.

In 1905 Dr. Pio Mingazzini published an account¹ of a gephyrean worm taken at a depth of 4,500 meters during an expedition of the Duke of Abruzzi in the neighborhood of Aukland. It is described as a spherical transparent organism, of about 6 mm. diameter, with a decided resemblance to certain sipunculid larvæ. However, as the author considered it to be an adult, it was placed in a new genus for which he states a new family may have to be created.

Spengel² chiefly on the basis of *Sipunculus* larvæ taken in the Bay of Naples, declares without hesitation that the animal in question is a larval form differing in a few minor details from other well-known types. The so-called gonad may well be the glandular appendages of the oesophagus noted in the young of several species.

During the past year two individuals, belonging to this proposed genus, have been taken in the surface plankton of Monterey Bay, Cal., and there is no doubt they are immature, the "gonadi" being, as Spengel surmised, glandular appendages of the pharynx. Nevertheless they present a number of features of considerable interest that are herewith described in some detail.

Both, apparently belonging to the same species, are spherical and measure 2.5 and 3.2 mm. respectively. In life the body wall was almost transparent, the muscles, especially those radiating from the mouth opening, appearing much as they do in *Doliolum* or *Salpa*. The alimentary canal was light yellow and the nerve cord and especially the brain were more opaque and fairly distinct.

The most prominent structure of these animals is the alimentary canal, which in its general features resembles that of the

¹ "Un Gefireo pelagico: *Pelagosphara aloysii* n. gen. n. sp.," *Rendic. Accad. Lincei, cl. sc. fis., mat. nat.* (5), Vol. 14, pp. 713-720.

² "Eine verkannte *Sipunculus*-Larve," *Zool. Anz.*, Bd. 31, 1907

species studied by Mingazzini though there are some important organs that have remained undescribed. The mouth (*m*) opens into a gradually enlarging cavity, lined with a continuation of the cuticle surrounding the body as far as the opening of the ventral glands. At this point a large pouch-like diverticulum (*d*) is developed chiefly on the dorsal side of the gut, and the naked cells of its lining epithelium are relatively high and slender and bear an excessively heavy coat of cilia at least two or three times their own length. In his figures Mingazzini represents six large retractors and a few smaller strands attaching to this point

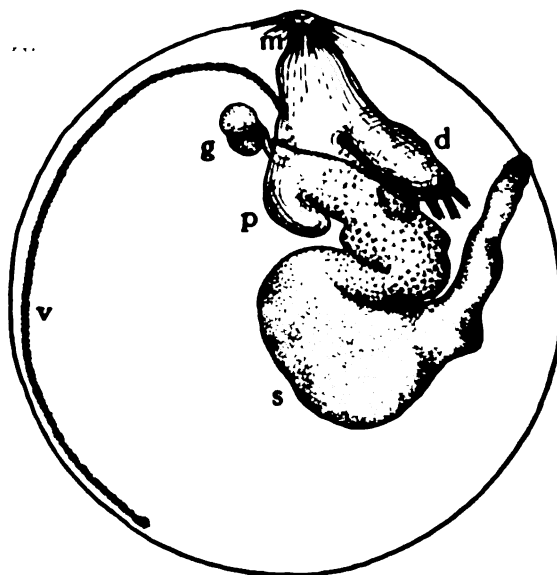


FIG. 1. Larval gephyrean (*Palagospira*). The stippling of the gut is merely to differentiate the different divisions. *d*, dorsal ciliated diverticulum; *g*, glands and duct, slightly displaced; *m*, mouth; *p*, ventral non-ciliated pouch or pharynx; *s*, stomach-intestine; *v*, ventral nerve cord leading to brain partially concealed by pouch *d*.

(though there is no sign of a pouch) and on the other hand radiating outward to become united to the body wall. In the present specimens the same is true. It appears probable therefore that this ciliated diverticulum may be everted and act as a locomotor organ as Wilson¹ has described in the case of *Echiurus*

¹ "Our North American Echiurids," *Biol. Bull.*, Vol. 1, 1900.

pallasii. And furthermore the proboscis or everted portion would, owing to its dorsal position, form a protuberance on the dorsal side of the mouth which would be situated, as in *Echiurus*, at its base. Perhaps to facilitate the eversion of this part of the digestive tract another outpouching (*p*) occurs on the ventral side of the gut, but its cells though columnar lack the ciliated coat, and it is without special retractor muscles.

This reference to *Echiurus* does not necessarily prejudice one in favor of the belief that the larvæ in question belong to this or allied genera. Setæ are totally lacking, there are no signs of segmentation, and so far as may be seen there are no cilia on the external surface of the body as in other gephyrean larvæ. For the present, at least, the question of systematic relationships cannot therefore be definitely determined.

Ventral to the gut in this same region are two spheroidal glands (*g*) in close contact with each other. In life they bear a fairly close resemblance to ova, which doubtless led Mingazzini to describe these organs as the gonad. Each is composed of a mass of pyriform cells, densely packed with a finely granular secretion from which ductules extend toward the inner face of each gland. A small duct, opening into the pharynx or œsophagus, extends anteriorly a short distance, and then bending ventrally it passes between the two glands whose ductules unite with it.

Beyond this point the digestive tract pursues an irregular course, and bending on itself passes dorsally to the anal opening. Three distinct divisions are clearly defined, the œsophagus, stomach-intestine and rectum. The first of these is of irregular outline, of relatively large caliber and its walls throughout are composed of columnar cells bearing a coat of delicate cilia. In the region of the stomach-intestine it narrows considerably and its walls become longitudinally folded. The stomach-intestine (*s*) is at first a comparatively large sac with highly glandular walls, each gland cell pyriform with the swollen distal end distended by some lightly staining, vacuolated secretion. Diatoms occur in this section of the tract together with small quantities of some other material, all of it enveloped in a stringy looking coagulum. Although the gut is narrowed for a considerable dis-

tance before the rectum is reached, and appears in whole mounts to be differentiated into a definite intestine, sections show that this same glandular epithelium extends from oesophagus to rectum. This latter structure is characterized by longitudinally folded walls lined with cuticle and provided near the anal opening with a strong sphincter muscle.

The nervous system is free from the ectoderm and is constructed upon the usual geophyrean plan. Posteriorly the nerve cord terminates in a slight enlargement, as noted by Mingazzini, and on the other hand, after following the body wall to a point close to the pharynx it bends inward and in the neighborhood of the duct of the ventral glands bifurcates to form the oesophageal collar. The course of these connectives in two or three sections is difficult to follow, owing to their small size and the abundance of connective tissue and muscle fibers, but the plan shown in the figure is probably not far astray. The bilobed brain is very distinct in both sections and in whole mounts, but it has been impossible to trace any nerves from it with the exception of the connectives.

The kidneys are two in number and consist of short coiled tubes opening to the exterior. I have examined these organs with much care but have failed to find that they have any internal opening. In every case the two extremities are in close contact, but the glandular portion includes the distal extremity, apparently, and if any inner pore does exist it is minute and not a well-developed nephrostome. The gland cells are fairly well defined, more or less vacuolated and contain considerable quantities of a finely granular secretion. In the neighborhood of the pore the glandular elements disappear, the canal becomes of much smaller caliber and after a short, sharp twist opens to the exterior.

The fatty substance described by Mingazzini is lacking in the present specimens, though in all probability this may be a variable feature. Also no trace of sex cells has been found, and there is therefore no reason to consider this an adult form so that a generic name is uncalled for as Spengel maintains.

BIOLOGICAL BULLETIN

THE DETERMINATION OF DOMINANCE AND THE MODIFICATION OF BEHAVIOR IN ALTERNATIVE (MENDELIAN) INHERITANCE, BY CONDITIONS SURROUNDING OR INCIDENT UPON THE GERM CELLS AT FERTILIZATION.¹

WILLIAM LAWRENCE TOWER.

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INTRODUCTION.

The Point of View.—The mutation theory of DeVries, the rediscovery of Mendel's paper on hybrid peas, and the work of the Mendelian hybridologists have focused the attention of biologists upon the characters of organisms as basal units for investigation in the effort to understand the *modus operandi* of evolution. The most conspicuous outcome is the growth of the "unit character" hypothesis, which, in the main, has been adopted by the Neo-Mendelians as the fundamental assumption necessary

¹A paper presented at the meeting of the American Naturalists in Boston, Dec., 1909.

for their practice and theory, because of the fact that the characters of organisms often stand out sharply and behave with distinctness in the processes which follow reproduction.

That many of the characters in organisms are distinct and behave in the sharp, alternative fashion described by the Mendelians, there can be no reasonable doubt. To deny the existence of these sharply defined, predicable behaviors in inheritance is to deny the evidence of our senses, and accuse a considerable body of reputable workers of inability to make accurate observations. *The fundamental question is, do these unit characters, or lesser entities, occupy in the organism the mosaic relation and have the capacity for the mosaic rearrangement which is assumed by most Mendelians and by the followers of De Vries?*

We must not be diverted from the main question by any fancied injury to our biological orthodoxies by the Neo-Mendelians' array of factors, determiners, allelomorphs, gametic couplings, latencies, etc., *because these represent only the symbols of processes at present unknown, although the results of these unknown processes are observable, predicable, capable of control and modification.*

Many, unable to accept the consequences which they believe to be the logical outcome of the unit character conception and the mosaic composition of organisms, take refuge in speculations in which the organic individual is compared to inorganic crystals, and state their position as follows:

In the inorganic world the attributes and qualities of a particular substance are due to the interaction of the component molecules and atoms, resulting in manifestations of one kind or another, which, as far as the mass is concerned, are essentially equivalent to the characters found in organisms. That is to say, in a crystal of copper sulphate the crystalline form and blue color are not due to a series of representative particles, or to unit characters, but they are due to the arrangement of the component molecules of copper sulphate and water of crystallization; in short, to a host of complex interactions of the component molecules. Copper sulphate crystals, in order to retain their specific identity, must consist of a definite proportion and arrangement of these molecules, and there is no *a priori* reason why it is any

different in organisms, unless we assume that there is in living substance something fundamentally different from that of non-living matter, and any such admission at once commits the maker thereof to vitalism.

I am not convinced that the analogy between a crystal and an organic individual is justified. This idea, of which Haeckel is the foremost exponent—while it has points in its favor—leaves much unexplained, and in living substance there are many processes and attributes which can be taken away, *i. e.*, are absent as far as perception is concerned, leaving a similar mass with other attributes.

There is much justification for considering organic species in the same light as we view non-living species. Thus a granite rock is as distinctive and specific as any animal or plant species. It has properties, attributes of the whole and also specific characters of the component parts. The basal components—orthoclase-feldspar, mica, quartz—exist in crystalline form in a granular crystalline complex; while the whole has specific attributes and qualities, the products of the interactions of the component parts and of the forces incident upon the elementary component substances at the time of combination. Depending upon variations of the mica—whether muscovit or biotite—and upon the amount and size of the mica masses, the appearance and attributes of the granites change; and much also depends upon whether the feldspar is orthoclase-feldspar or triclinic-feldspar, etc.

The possible number of specific kinds of granite is very considerable and depends solely upon the nature of the component substances and the conditions under which the combinations are effected, while the specific end products are as distinctive as any plant or animal form.

Likewise in feldspar, (a rock-forming unit character?) the crystalline form depends upon what it is that is combined with the silicates of aluminum, whether salts of calcium, sodium, potassium or barium, to give the range of color, form, hardness, cleavage, specific gravity, etc., found in the feldspar. Feldspar is crystalline, has polarity, and might well be a parallel to an organic form, but the constituent substances can be changed, replaced, giving distinct, specific end results. In orthoclase, which

is a potash feldspar, is the potash a mineralogical unit character? It seems to me that the problem of the constitution of natural substances is the same in both living and non-living substance, and that there is in living substance something very much like that which is found in non-living substance in the combining of attributes from diverse sources into compact and distinct wholes.

Because a character is sharp and distinct, or may be apparently removed, is no necessary reason for supposing that it may be taken away as an entity, and no reason whatsoever for believing it to be conditioned by a subsidiary mass; rather, we must regard an organism essentially as we would look upon any inorganic substance, as a mass of matter in which the unit is the individual with its array of attributes or qualities, or its specific characters. It is certain, however, that there is adequate evidence to prove the truth of the situation as described by the Mendelians as far as the behavior of the attributes is concerned; but the basis and cause of this behavior is entirely unknown, and must remain unknown until we have replaced by fuller knowledge the present crude ideas of the constitution of living substance.

The situation which has developed in the last few years at the hands of the Neo-Mendelians themselves is interesting.

From the simple conditions discovered by Mendel there has arisen through the work of the last decade an array of observations tending to show that the Mendelian phenomenon is not in many instances as distinct and simple as one might wish, and at present divers kinds of variability in the behavior of characters are described and attributed, in some instances, to several different kinds of latency, to gametic coupling, to variable potency, to variable dominance, and so on. The situation essentially is this, that as investigation has progressed it has been discovered that not one, but a host of determining factors (I use the word factor as meaning something which makes possible a given result, with no idea expressed or implied as to the nature of this factor) are operative in the production of alternative inheritance; and in the attempt to preserve the letter of the law of Mendelian theory of unit characters with segregation in gametogenesis, a host of hypotheses have been developed in order to save the original theory.

Among the Neo-Mendelians the assumption is universal that all the differences that come out of crosses are entirely due to internal factors brought into the fertilized egg by the gametes as factors and determiners, to various combinations of allelomorphs, and so on. That some of these variable conditions may be due to external causes does not seem to have occurred to any of the Mendelians, and no effort has been made to eliminate in any one case what would first be eliminated in any accurate physical or chemical work, namely, the effect of surrounding conditions, and forces incident upon the reactions unquestionably going on in the developing individual.

Probably most of us will admit that the fertilization process represents the bringing together of two more or less like physico-chemical masses, and the combining of these into a new body with potentialities and capacities which then enable it to go on in a constantly increasing series of epigenetic reactions, and ontogenetic processes, and finally to evolve into an adult organism. The essence of the activity and reaction involved are beyond any question physico-chemical in their nature. This being true, the first step in the elucidation of this complex array of variability in behavior is to determine to what extent surrounding or incident forces may modify in a particular case the type of alternative inheritance which is found; when effects of these forces are known, then attack can be profitably made upon problems of internal factors. This first step I have in part accomplished in a series of experiments which form the basis of this preliminary paper.

Material.—The material upon which this series of experiments herein described was carried out consisted of three species of chrysomelid beetles of the genus *Leptinotarsa*, which would hybridize freely and perfectly in all directions: *Leptinotarsa signaticollis* Stål, a species occurring in southern Mexico at the foot of the escarpment on the western side of the Mexican plateau; *Leptinotarsa undecimlineata* Stål, a species confined entirely to the savannahs and lower foot hills from Tampico in Mexico, southward to Costa Rica and Panama; and *Leptinotarsa diversa* n. sp., which is very similar to the former but is limited entirely to the higher foot hills on the border of the Mexican plateau. The reason for

choosing these three species was that any two of them, when crossed, give only certain very definite and invariable products, and I was thus enabled to eliminate many complications and possible sources of error. The contrasting characters are as follows:

Between *L. signaticollis* Stål (Fig. 1) and *L. undecimlineata* Stål (Fig. 2), in the adult, the elytra of *L. undecimlineata* have deep greenish black longitudinal stripes, edged with a double row of punctations, while *L. signaticollis* has the punctations, but not the stripes. The ground color of the elytra in *L. signaticollis* is grayish, while in *L. undecimlineata* it is white. No other characters in the adult had sufficient contrast to give sharp alternative inheritance. The larvæ of the two are sharply contrasted; those of *L. undecimlineata* and *L. signaticollis* are exactly



FIG. 1. *L. signaticollis* Stål. (A) Adult. Showing the absence of pigment, and the presence of the impressed punctations on the elytra which border the stripes in other species. The absence of pigment may well represent the negative half of a Mendelian allelomorph. (B) Full-grown larva. Showing the arrangement of black color marks upon the sides and back of the larvæ. The ground color in this state is bright chrome yellow. (C) Larva of second stage. Showing the characteristic color pattern. The ground color is a light chrome yellow.

alike in the first stage, but in the second stage *L. undecimlineata* is white and *L. signaticollis* yellow, both with the same system of spots, and in the third stage *L. signaticollis* is yellow with well-developed tergal stripes and *L. undecimlineata* is pure white without stripes. These differences are well shown in text Figs. 1 and 2.

In *L. diversa* (Fig. 3) the elytra are marked by longitudinal stripes of greenish black edged with an irregular double row of punctations, and the larvæ are in all stages exactly like those of

L. signaticollis. There are no other simple differences between the three species that could be readily utilized in experiments of this kind, but the characters used are sharp and striking, and satisfy in every respect the conditions demanded by Mendelian hypotheses.



FIG. 2. *L. undecimlineata* Stål. (A) Adult. Showing on the elytra the presence of longitudinal pigmented bands which are bordered by rows of punctations as in *L. signaticollis*. The pigmented bands may be considered the positive half of a Mendelian allelomorph, so that when the two species are crossed we are following all of the Mendelian qualifications in crossing the presence and absence of the same character. (B) Full-grown larva, showing the characteristic color pattern in this stage. The ground color is pearly white. (C) Second stage larva, showing the characteristic color pattern. The ground color is also pearly white.

The elytral stripes of *L. diversa* and *L. undecimlineata* represent the present or positive character, and the lack of stripes in *L. signaticollis* the absence or negative factor of an allelomorphic pair, the yellow hypodermal color one member and the white the other of an allelomorphic pair: $yl = (+)$ $wh = (-)$; the dorsal spots the presence $(+)$ and their absence the absence $(-)$ of another pair of allelomorphs—when we view the characters from the present Neo-Mendelian standpoint.

A further reason for choosing this material for this investigation is the fact that when crossed it often gives most perfect Mendelian results, and in some cases perfect ratios of 1:2:1 are obtained in the second hybrid generation, while other crosses, brothers and sisters of the same material, did not give the same, but on the contrary, quite different results. For some time I was at a loss to understand the reason for this anomalous condition. No amount of crossing or investigation succeeded in disclosing

the presence of any internal factor, which by its operations would produce this result. Careful examination, however, of the records which have been kept in the vivarium where these experiments were carried on, gave evidence that the differences were possibly due to the conditions surrounding the hybrid series during development. Accordingly, an array of experiments has

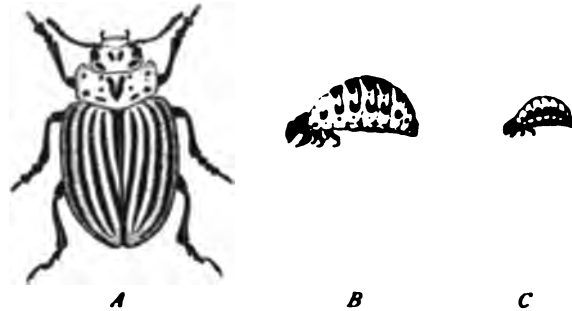


FIG. 3. *L. diversa*. (A) Adult. Showing the presence on the elytra of longitudinal dark stripes in exactly the same position as the dark stripes in *L. undecimlineata*, and in the position of the absence of stripes in *L. signaticollis*. (B) Full-grown larva, showing characteristic color pattern. The ground color is bright chrome yellow as in *L. signaticollis*. (C) Second stage larva, showing characteristic color pattern. The ground color is yellow.

been carried out to test this point—do the conditions surrounding or incident upon the gametes before and at fertilization and in early ontogeny influence in any way the behavior of characters? As far as this phase of the modifiability of alternative inheritance is concerned, I have essayed to investigate it in two ways: first, by the usual process of hybrid analysis, as it is practiced in all Mendelian work, in which the extracted dominants, recessives and heterozygous forms are isolated and carried on as pure cultures, and again inbred and tested in the usual ways—essentially experiments in analysis to determine germinal constitution; second, I have carried on a series of complicated experiments in synthesis, comparable to those which one would expect to go on in nature when two of these species are brought into contact and hybridized, in order to learn what the result would be if two species should come in contact under different conditions, and hybridize, and these results I shall describe under the head of Experiments in Synthesis.

EXPERIMENTS IN ANALYSIS.

Crosses Between L. signaticollis and L. diversa.

The first effort was to determine whether or not external or incident conditions were in any way responsible for the production of the diversity of behavior found. The first experiment was a cross between a male *L. diversa* and a female *L. signaticollis*.

L. signaticollis ♀ × ♂ *L. diversa*.

Exp. No. H 409.¹

A virgin female of *L. signaticollis*, from Exp. No. 419, g. XI (CCB) was mated with a male *L. diversa*, from Exp. No. 816, g. VI (A), under the following conditions:

FOOD : NORMAL — UNIFORM.

T.		R. H.	
Day Av.	80° F. ± 5°.	Day Av.	75 per cent. ± 5 per cent.
Night Av.	75° F. ± 8°.	Night Av.	75 per cent. ± 5 per cent.

In the F₁ generation the larvæ were all alike, as was to be expected since the larval stages of the parental stocks are similar. These larvæ gave rise to F₁ hybrid adults distributed in two sharply marked groups. One of these groups was indistinguishable from the female parent stock, and the other was an intermediate between the male and female parental stocks, in which the essential contrasting character between the adults was in the elytral stripes and these were distinctly midway between the two parent types, (a blend). The numbers were 49 of the *signaticollis* type (sig. type), to 53 of the mid type (mid type)—practically a 1:1 ratio.

Several pairs of each of these types were mated for the F₂ generation. The matings of the *signaticollis* type gave in the F₂ generation pure *signaticollis*, and further breeding for five consecutive generations gave only *signaticollis* types. The progeny of the mid type, however, split in the F₂ generation into three classes, with perfect Mendelian ratios, one like the female *signaticollis* type, one like the male *diversa* type, and the mid type. For example, pair A, gave:

sig. type.	mid. type.	diversa type.
observed 59 :	121 :	61.
expected 60.25	120.50	60.25

¹ These experiment numbers are those of the original records, which are now attached to the specimens preserved as a record of this work.

The extracted forms of the *signaticollis* type, when inbred, continued to breed true in F_3 ; the extracted forms like the male *diversa* type likewise came true in F_3 , F_4 and F_5 ; and the mid types in generations F_3 and F_4 gave in all cases close approximations to the ratio 1:2:1. The behavior observed in experiments of this class is shown in Plane I., a digest of Exp. No. H 409.

This behavior would suggest that either one or the other of the parents used was a "heterozygous form." When, however, identical material, brothers and sisters of the first, were reared under other conditions, a different result was obtained.

L. signaticollis ♀ × ♂ *L. diversa*.

Exp. No. H 410.

In Plate II. are represented the results obtained in crossing a female *L. signaticollis* and a male *L. diversa* of exactly the same stocks as described in Exp. No. H 409, but under the following conditions:

FOOD : NORMAL — UNIFORM.

T.		R. H.	
Day Av.	75° F. + 5°.	Day Av.	50 per cent. ± 10 per cent.
Night Av.	50° F. ± 5°.	Night Av.	80 per cent. ± 15 per cent.

In the F_1 generation of this experiment there was one class of adults, invariably of the mid type, and these, when inbred, gave in the F_2 generation a typical Mendelian grouping of types, in the majority of cases in as nearly perfect ratio of 1:2:1 as could be desired. These two crosses were repeated eleven times under the same conditions with the same results, the only variation being a slight oscillation in the 1:2:1 ratio of the F_2 generation and a slight variation in the mid types. The number of pairs that have been used would in itself be sufficient evidence that the results were not due to the chance selection of individuals which would give these results, but that they were due entirely to the conditions surrounding the germinal materials at the time of their combination and during their early development. To still further test the point, a more crucial experiment was carried out as follows:

L. signaticollis ♀ × ♂ *L. diversa*.

Exp. No. H 409/411.

A female of *L. signaticollis* and a male *L. diversa* were allowed to reproduce under the conditions of the first experiment (Exp. No. H 409), while a certain number of their eggs were being laid (designated as Lot A). These eggs developed normally under the following conditions:

FOOD : NORMAL — UNIFORM.

T.		R. H.	
Day Av.	80° F. ± 5.4°.	Day Av.	74 per cent. ± 6 per cent.
Night Av.	75° F. ± 7.8°.	Night Av.	75 per cent. ± 5.7 per cent.

and gave typical larvæ, which gave two classes of adults in the F₁ generation in the proportion of approximately 1:1, or

sig. type.	mid type.
88 :	94.

The individuals like the female parent (sig. type), when inbred, came true to the type for four consecutive generations, which was as far as they were carried; while the mid types, when inbred, gave in the F₂ generation marked Mendelian ratios in the proportion of 1:2:1, the numbers for Pair B being:

<i>signaticollis</i> type	mid type.	<i>diversa</i> type.
Observed 98 :	201 :	101.
Expected 100 :	200 :	100.

After a few days the same pair of individuals was placed under the conditions of Exp. No. H 410:

FOOD : NORMAL — UNIFORM.

T.		R. H.	
Day Av.	± 75° F. 4.3°.	Day Av.	51.2 per cent. ± 11 per cent.
Night Av.	± 51° F. 5.8°.	Night Av.	81.5 per cent. ± 14.2 per cent.

and there allowed to develop another set of eggs (Lot B).

These eggs developed normally and gave in the F₁ generation only a single type like the *signaticollis* parent, which, when inbred, gave in the F₂ generation *signaticollis*, and continued to breed true for four consecutive generations. This experiment was repeated seven times with uniform results, confirming the

conclusions drawn from the first two experiments and showing beyond doubt that the variability of behavior in the alternative inheritance of the elytral stripes in these two species of beetles was due to conditions surrounding and incident upon the germinal materials in their most sensitive stages, before, during and immediately following fertilization. The behavior common to this type of experiment is shown in Plate III.

With this cross of an *L. signaticollis* ♀ × ♂ *L. diversa*, the determination of dominance and the ensuing type of behavior is clearly a function of the conditions incident upon the combining germ plasms. In the repetition of Exp. No. H 409/411, the experiment was varied so that in some cases it was the first laid eggs that gave the behavior of Exp. No. H 409, and in others, it was the last laid eggs, or those of the middle of the reproductive period—showing that the results are not "age results," nor due to segregations, nor orthogenesis giving one kind of germ at the start, another at the middle, and others at the close of the reproductive period.

Crosses Between L. signaticollis and L. undecimlineata.

More interesting and complicated results were obtained in crosses between *L. signaticollis* and *L. undecimlineata*, where there are contrasting characters between both larvæ and adults, differences in the specific pattern as a whole, in specific spots and in the general body color. Reference to Figs. 1 and 2 will show essentially what these differences are, as far as pattern and spots are concerned. In body color of the larvæ the difference is between a white in *L. undecimlineata* and a bright chrome yellow in *L. signaticollis*.

L. undecimlineata ♀ × ♂ *L. signaticollis*.

Exp. No. H 700 A.

A virgin female of *L. undecimlineata*, from Ex. No. 722, g. III (AA) was crossed with a male *L. signaticollis* from Ex. No. 419, g. XIII (CAAC) under the following conditions:

FOOD : NORMAL — UNIFORM.

T.		R. H.	
Day Av.	+ 95° F. 3.5°.	Day Av.	84 per cent. + 8 per cent.
Night Av.	+ 89° F. 4.8°.	Night Av.	100 per cent.

This mating gave larvæ in the F_1 generation which were of the female parent type, *i. e.*, white without tergal spots in the third stage, and from these emerged a single type of adults, all of the female parental type. These, when inbred, came true, giving individuals like themselves, and continued to breed true for six generations. No traces of sporadic variations of any kind in either adult or larva were found, nor was there in this strain any trace of the male parental type. In this cross, which I have repeated five times, the male type was as completely eliminated as if it had never existed. Plate IV. shows the behavior in the first and second hybrid generations.

Question as to this result might be raised on the grounds that it is a case of parthenogenesis and not a case of hybridization at all. As a matter of fact, parthenogenesis in *Lep. tinotarsa* is unknown, and although I have repeatedly endeavored to obtain parthenogenetically developed individuals, I have always signally failed. Virgin females of *L. undecimlineata* have been repeatedly subjected to chemical stimuli (*i. e.*, injected salts of Cu, Na, Li, Sr, Ba, Cu, Zn, etc.), and physical stimuli (*i. e.*, mechanical shocks, electric stimuli, etc.), which might be productive of parthenogenetic development, but thus far without any success whatever.

In a second series of experiments, an exact duplicate of the first, a female was allowed to develop eggs and deposit them, and these eggs uniformly failed to develop even to the earliest cleavage stages. No development and no fertilized eggs were found from this female until the eggs were fertilized by a male *L. signaticollis*, when development progressed in the regular manner and gave results identical with those of the first experiment.

L. undecimlineata ♀ × ♂ *L. signaticollis*.

Exp. No. H 700 A.

A virgin female of *L. undecimlineata*, from Exp. No. 722, g. III (AB), was crossed with a male *L. signaticollis*, from Exp. No. 419, g. XIII (CAAC), under the following conditions:

FOOD : NORMAL — UNIFORM.

T.		R. H.	
Day Av.	75° F. ± 2° .	Day Av.	80 per cent. ± 5 per cent.
Night Av.	70° F. ± 3.5°.		80-90 per cent. ± 5 per cent.

From this cross there came larvæ which in the F_1 generation were all of the female parental type, and from these emerged a single class of adults intermediate between the two parents, a mid type. These adults, very uniform in character, when inbred, gave in the F_2 generation abundant progeny. The larvæ of the F_2 generation were in the first stage all alike; in the second stage they were clearly divisible into two classes in the proportion of white 245: yellow 190; while in the third stage the yellow larvæ further divided into larvæ which were yellow with black spots on the back (YIS), and yellow without black spots on the back (YIs). The white larvæ similarly divided into two classes, white with black spots (WhS), and white without black spots (Whs). The census of larvæ in the third stage gave for Pair C:

Whs	WhS	YIS	YIs
170	75	58	132

Each of these classes of larvæ was somewhat variable, and it was often difficult to decide in the case of a white larva whether it belonged in the class with black spots or in the class without black spots, because there is an almost continuous gradation of color from zero to a full development of the dorsal color pattern. In making the division, the practice was followed of examining the larvæ with a lens magnifying ten diameters, and if the slightest trace of pigment was detected in the centres in which pigment develops, they were then classed as larvæ with spots, even though the spots were not visible to the unaided eye.

Each of these four classes of larvæ gave rise to three classes of adults, that is, each one split into forms like the female type, like the male type, and an intermediate type. In every case each of the four classes has given a typical Mendelian splitting in the second hybrid generation, but the ratios are in most cases somewhat variable, owing to the necessity of breeding these F_2 hybrids under rather variable conditions. The behavior in this culture is shown in Plate V. The census of the adults derived from Pair C was as follows:

	11-lineata type.	mid type.	sig. type.
170 Whs larvæ gave:	14♂ 22♀ 36	24♂ 25♀ 49	4♂ 7♀ 11
75 WhS larvæ gave:	3♂ 8♀ 11	9♂ 11♀ 20	6♂ 4♀ 10
58 YIS larvæ gave:	4♂ 4♀ 8	6♂ 10♀ 16	5♂ 4♀ 9
132 Yls larvæ gave:	9♂ 7♀ 16	22♂ 25♀ 27	17♂ 15♀ 32

Ratios of these adult types	71 :	132 :	62 :
Expected	63.75	127.50	63.75

Four experiments of this type have been carried through to the fourth and fifth generations with perfectly uniform results thus far.

L. undecimlineata ♀ × ♂ *L. signaticollis*.

Exp. No. H 701.

A virgin female of *L. undecimlineata*, from Exp. No. 722, g. IV (E), was crossed with a male *L. signaticollis*, from Exp. No. 419, g. XIII (CAAB) under the following conditions:

FOOD : NORMAL — UNIFORM.

	T.	R. H.
Max.	105° F.	Max. 85 per cent.
Min.	80° F.	Min. 70 per cent.
Av.	92° F.	Av. 79 per cent.

The larvæ of the F_1 generation were in the first stage identical; in the second stage they were clearly divisible into sharply marked classes of white and yellow in the proportion of wh. 30:yl. 26. The yellow larvæ in the third stage divided into YIS 11 and Yls 9, and the white larvæ gave three groups: pure pearly white like the female parent, whs 1; larvæ which were yellowish white without spots, whys 14; and larvæ which were yellowish white with spots, whyS 10. The dorsal spots of the larvæ in the third stage were in this case variable. The single pearly white larva gave rise to a female exactly like the parent type and four classes of larvæ: white without spots (Whs), white with spots (WhS), yellow without spots (YIS), and yellow with spots (Yls). Each gave rise to a group of highly variable mid types, in which the range of variation was from the condition of the female parent to that of the male parent. Fourteen pairs of these were tested out in the F_2 generation and they were all found to be uniformly

mid types, that is, they were "heterozygous" in their nature and every one of them gave, when inbred, the following behavior in the F_2 generation: the larvæ in the first stage were all alike, in the second stage they were clearly divisible into two types, white and yellow in a proportion of approximately 1 : 1; in the third stage the larvæ of all the mid types divided into four classes: white without spots (Whs), white with spots (WhS), yellow without spots (YIS), and yellow with spots (YIs); and each of these classes gave three classes of adults, that is, from the white larvæ without spots came forms which were exactly like the female parent, forms exactly like the male parent, and an intermediate type. The same classes of adults came from the white larvæ without spots, from the white larvæ with spots, from the yellow larvæ with spots and the yellow larvæ without spots, giving thus twelve sharply marked categories of adults in the second hybrid generation, each having a definite type of behavior and carrying presumably a definite gametic constitution, the product of its past experience. The census of larval characters from nine matings was as follows:

	Whs.	WhS.	YIS.	YIs.	Total.
Pair A.	110	39	28	53	230
Pair B.	233	92	30	71	426
Pair C.	248	83	65	116	512
Pair D.	92	72	44	51	259
Pair E.	107	64	40	31	243
Pair G.	111	29	57	102	399
Pair I.	70	22	21	52	165
Pair K.	170	75	58	132	435
Pair L.	271	69	70	164	574
Total.	1,411	545	413	772	3,243

From these larvæ there came the three types: *signaticollis* (sig.), mid (mid), and *undecimlineata* (11-lin.) as follows:

	Whs. Larvæ.			WhS Larvæ.			YIS Larvæ.			YIs Larvæ.		
	11 lin.	mid	sig.	11 lin.	mid.	sig.	11 lin.	mid.	sig.	11 lin.	mid	sig.
Pair A.	15	34	14	5	14	6	2	8	10	7	21	12
Pair B.	39	43	20	13	35	5	3	10	5	6	28	9
Pair C.	43	72	32	20	26	9	10	27	10	7	26	12
Pair I.	14	36	7	4	8	6	1 ¹	8	7	1	28	13
Pair K.	36	49	11	11	20	10	4	16	9	16	47	32
Total.	147	234	84	53	103	36	20	69	50	37	150	78

¹A hybrid sport ♀ with the elytral stripes double — because the ancestor of a stable race

OR

	11-lin.	mid.	sig.
Observed.	257 :	556 :	288.
Expected.	275 :	550.50 :	275.25.

These have been carried on into subsequent generations, and it has been found that the twelve extracted types resulting in the second hybrid generation are different from each other, even though they may appear superficially alike. The general behavior and results obtained in this experiment are shown in Plate VI.

L. undecimlineata ♀ × ♂ *L. signaticollis*.

Exp. No. H 700.

A virgin female of *L. undecimlineata*, from Exp. No. 722, g. I, was crossed with a male *L. signaticollis*, from Exp. No. 419, g. XI (DC), under the following conditions:

FOOD : NORMAL — UNIFORM.

T.	R. H.
Max. ± 81° F.	Max. ± 87 per cent.
Min. ± 70° F.	Min. ± 69 per cent.
Av. 75.6° F.	Av. 77.11 per cent.

This cross gave rise in the F_1 generation to larvæ all alike in the first stage, which in the second stage separated into two classes, yellow and white, and in the third stage remained as two classes, white without spote (Whs) and yellow without spots (Yls) (Whs 37 : Yls 45). The white larvæ (Whs) gave rise to twenty-one individuals exactly like the female type (12 ♂, 9 ♀), and the yellow without spots (Yls) gave rise to twenty-four individuals of the mid type (11 ♂ 13 ♀), a ratio of 7 : 8. The individuals like the female parental type, when inbred, gave a typical repetition of the ontogeny of the female parental stock with adults like themselves, and continued to do so for four consecutive generations without any indication of the male parent type appearing. The mid type, when inbred, gave in the F_2 generation typical results. The larvæ of the first stage were all alike; they divided into yellow and white larvæ in the second stage in the

proportion of yl. 83 : wh. 154, and in the third stage the white larvæ split into

Whs.	WhS.	YIS.	Yls.
38:	33:	31:	20.

or approximately 4 : 2 : 2 : 1. Each of these groups then gave rise to three classes of adults, precisely similar to those found to arise in the second hybrid generation in Exp. No. H 701, that is, from each of the four larval groups there arose forms like the female parent, others like the male parent, and an intermediate form; and these three categories of adults are clearly distinguishable one from the other without intermediates between them. The general results and behavior of this experiment are shown in Plate VII.

L. undecimlineata ♀ × ♂ *L. signaticollis*.

Exp. No. H 701 B.

A female *L. undecimlineata*, from Exp. No. 722, g. IV (E), and a male *L. signaticollis* from Exp. No. 419, g. XIII (CAAB), were crossed under the following conditions:

FOOD : NORMAL — UNIFORM.

T.	R. H.
Max. ± 98° F.	Max. + 95 per cent.
Min. + 59° F.	Min. + 40 per cent.
Av. 80.2° F.	Av. 68 per cent.

and gave rise in the F₁ generation to larvæ which were all alike in the first stage, but which separated into yellow and white in the second stage, in a proportion of yl. 21 : wh. 28; the white larvæ remained white larvæ without spots in the third stage and the yellow larvæ remained yellow larvæ without spots in the third stage.

From the white larvæ without spots there came a type exactly like the female parent (2 ♂, 2 ♀), which, when inbred, continued to breed true; and from the yellow without spots came two classes of adults, a type exactly like the male parent (3 ♂, 2 ♀), and a mid type (3 ♂, 4 ♀); of the three types appearing in the F₁ generation, which are sharply marked one from another without any trace of intermediates, the observed numbers were:

11-lin. type.	mid type.	sig. type.
4 :	7 :	5.

In this experiment in the F_1 generation three distinct types appeared, one like the male parental type, one like the female parental type, and a mid type. Each of the derived types was inbred for four consecutive generations and continued to breed true, neither one giving any indication of the other stock. Of the mid types several pairs were mated and gave the same results as those obtained from the mid types in Exp. No. H 700, H 701, etc. Thus, in Pair A, the larvæ in the first stage were all alike; in the second stage they split into white and yellow in a ratio of wh. 55 : yl. 45. The white split into white without spots (Whs 27), and white with spots (WhS 23); and the yellow split into yellow without spots (Yls 14), and yellow with spots (YIS 25). The four classes of larvæ gave adults of the F_2 generation, each producing three classes sharply demarked one from the other, as in the previous experiments. Plate VIII. shows the behavior and results obtained in this experiment.

In this series of experiments the behavior in the first and second hybrid generations indicates a variability in the reactions which take place between the combinations brought about in the germ plasm at fertilization. As stated in the introduction, I have not been able to show the existence of additional characters latent or recessive, which might bring about the variability in behavior observed, and the problem is to account for this variability.

In the absence of evidence to the contrary, the characters seen may safely be assumed to represent the characters involved, and it is perfectly clear, in crosses between *L. signaticollis* and *L. diversa*, and between *L. signaticollis* and *L. undecimlineata*, that when identical materials were placed under dissimilar conditions, dissimilar types of behavior resulted without the production of anything new or unusual in the way of attributes in the resulting progeny. In the case of Exp. No. H 409, the behavior there strongly indicated that either one or the other of the two parents was heterozygous in character, and such an inter-

pretation would perhaps be perfectly valid were it not for the fact that identical materials placed under dissimilar conditions, as in Exp. No. H 410, give such a result that one would not be warranted in assuming that either of the parents was heterozygous, but that both parents were homozygous in the strictest sense. Even this might possibly be accounted for by assuming that chance had brought about the selection of homozygous individuals for one series of experiments and heterozygous individuals for the other. This interpretation, however, is invalidated by Exp. No. H 409/411, in which a male and a female of material identical with that used in Exp. No. 409 and Exp. No. 410 were allowed to reproduce under the conditions of Exp. No. 409 for part of their reproductive period, and under the conditions of Exp. No. 410 for the remainder. From the eggs developed and fertilized during the first period came results in every way comparable to those obtained in Exp. 409, and from the eggs developed and fertilized during the second period came results in every way the duplicate of those obtained in Exp. No. 410.

These experiments are crucial as far as this case is concerned, for the simple reason that it is inconceivable that during one period all the heterozygous germ cells would be developed, and during another period all the homozygous cells. Any such assumption is preposterous, and the only interpretation that one can legitimately use is that conditions surrounding and incident upon the germ cells at the time of and immediately following fertilization were in some way productive of the differences in behavior found.

In the series of experiments between *L. signaticollis* and *L. undecimlineata*, a more complicated behavior is found, due to the fact that more characters are involved. As in the first series, similar materials reared under dissimilar conditions gave dissimilar behaviors, but without the production of any new types or characters in the materials; that is, there are apparently no strange attributes present, but simply a variability in the behavior of attributes already present, and this variability in behavior may be of profound importance in evolutionary processes.

In this preliminary account of the experiments, I have given only the crosses between the female *L. signaticollis* and the males of *L. diversa* and the female *L. undecimlineata* and male *L. signaticollis*. The reciprocal crosses have been made and confirm in every way the results obtained in the crosses given, but in this paper the object is not the investigation of variability of behavior in reciprocal crosses, but an elementary consideration of the effect which incident or surrounding conditions may have upon the behavior of characters in hybridization. A more detailed treatment of this work must be postponed for a more extensive publication.

The results obtained in both sets of experiments are in general very much in harmony with results which have been obtained by certain workers in experimental embryology, with this difference, that in this case the analysis has been carried out through two or more generations, and in the case of experimental embryology the experiments cease with the obtaining of larval forms.

The whole question of dominance is at the present time a trying one. Practically all the criteria which have been proposed from time to time for the detection of the dominant member of a cross have in one way or another been broken down, and Davenport's definition, that the dominant member of a cross is only to be detected, in many organisms at least, by the determination of which character is the more variable in subsequent generations, seems to me not likely to be of general applicability. In the materials which form the basis of this paper it could not possibly be used, because both of the extracted forms are at times equally variable, at times one is more variable than the other, and at other times both are relatively invariable.

What determines the dominance of one parental type over the other is a question of vital importance in both normal and cross fertilization. Apparently, one of the earliest workers to attack this question was Vernon in 1898. He suggested that the hybrid echinoderm larvæ which in the summer were largely maternal in type, and in winter were largely paternal in type were due to the relative differences in the ripeness of the sexual products used in the cross.

Doncaster, in 1904, concluded that temperature was the chief,

if not the sole cause of the changes observed by Vernon; and Herbst's work in 1906-07 on the echinoderms showed definitely that temperature is a contributing, but not the exclusive factor. Tennent in 1910, by crossing *Hipponoë esculenta* with *Toxopneustes variegatus* at Dry Tortugas, Florida, reached the general result that in normal sea water *Hipponoë* is the dominant member of the cross in the *plutei*; and in sea water to which sodium hydrate had been added, increasing the alkalinity, he found that *Hipponoë* was also dominant irrespective of the direction of the cross; but when a small proportion of acetic or hydrochloric acid was added to normal sea water, thus decreasing the alkalinity, he found that *Toxopneustes* was dominant. This he considers an explanation of the seasonal variation described by Vernon, but it is not quite clear just what could be the basis of a seasonal variation of the alkalinity or acidity of a large body of ocean water like that of the Gulf of Mexico; rather, it is entirely conceivable, as Vernon states and as Herbst demonstrates, that temperature is at least a factor capable of producing variability in the dominance of various crosses. These results of Vernon, Doncaster, Herbst and Tennent deal with the early stages, and none have been carried to F_1 generation adults, or into the F_2 and F_3 generations. In my crosses exactly similar results have been produced by subjecting the organism to different sets of conditions during the fertilization period, and this has brought about divergence in behavior, which divergence has continued into the second and third generations, and the products of the variability are in every way constant and fixed from the start.

These results indicate that in the fertilization process the two somewhat unlike germinal substances that are being combined, and interact one upon the other in exactly the same way that two non-living substances would; that is, the products of the interaction are the resultant of the natures of the two substances and the conditions under which the combination took place. We must not, therefore, expect to find a factor which determines dominance, such as temperature, alkalinity, moisture, etc., but rather must determine the complex under which, when two materials are combined, definite

results are produced. When the same materials are combined under the conditions of some other complex we may expect, and do find, that there are differences in the behavior and in the products produced.

That this production of variability in behavior and in the determination of dominance by incident factors is a real and vital process in nature, I have attempted to show by a second series of experiments, in which I placed definite organisms together in a state of nature where they would interbreed freely, in order to see what the resulting products would be. These are the experiments in synthesis.

EXPERIMENTS IN SYNTHESIS.

The purpose of these experiments was to determine what the result would be when species which cross freely, and some of whose characters behave Mendelianwise in experiment, were brought together in a state of nature. It is frequently maintained, especially by systematists, that crossing in nature, while uncommon, when it does occur is without much effect—a broad statement made *à priori*, and grounded in the orthodox belief that species for the most part are physiologically isolated one from another.

An extensive set of these experiments has been in progress for some years, some few of which have now developed far enough to allow of rather definite statements. The method employed has been to take species derived from nature from some restricted locality, to keep close watch upon what goes on in this locality and also to analyze the composition of the species from this locality by cultures in the laboratory. In this way, stocks of known character are obtained from experiment, and also natural stocks whose attributes are well known are developed in the type localities. In the experiments in synthesis either pedigreed stocks from the laboratory, or the stocks from nature, or both, were placed in nature upon their food plant in isolated localities, or in large cages, and allowed to breed as if the introduction were a natural one.

COMPETITION HYBRIDIZATION EXPERIMENTS BETWEEN
L. SIGNATICOLLIS AND *L. UNDECIMLINEATA*.

L. undecimlineata × *L. signaticollis*.

Exp. No. C. H. 47.3 Cuernavaca.

In 1904, an isolated area of about an acre upon the southern slope of a barranca, near Cuernavaca, was planted with food plants, upon which both *L. signaticollis* and *L. undecimlineata* would feed. In July, 1904, this spot was stocked with a culture of 210 specimens of *L. signaticollis*, from a standard location about a mile and half distant, and 354 specimens of *L. undecimlineata*, obtained at El Hule, on the banks of the Rio Papaloapan. Each of the groups was equally divided between the sexes, were young and vigorous, immediately began breeding and intercrossed freely. Under experimental conditions these forms cross freely in both directions, but out of them no new characters come as the result of ordinary crossing.

In the first generation of this colony there was an abundance of individuals of both sexes of the *signaticollis* type, and of the *undecimlineata* type, and of a highly variable intermediate hybrid type. A census was made of the population on August 14 to 17, with the following results:

<i>signaticollis</i> type	mid type.	<i>undecimlineata</i> type.
4,518	11,744	5,091

In this experiment, it was, of course, impossible to tell from inspection whether the *signaticollis* individuals were pure *signaticollis*, or pure *signaticollis* and a hybrid with the *signaticollis* dominant, and the same was true with respect to the *undecimlineata*. All of the beetles entered into hibernation during the latter part of August, and early September of 1904. The food plants survived the long hard dry season and came up in the spring of 1905 in abundance, and in June, 1905, individuals of all three types emerged and were found to be interbreeding freely. A census made of the individual which emerged late in June gave the following results:

<i>signaticollis</i> type.	mid type.	<i>undecimlineata</i> type.
1,027	1,744	478

which clearly indicates that through some cause the hibernating conditions of the location were favorable for *signaticollis*, but were decidedly unfavorable for the *undecimlineata* and for the intermediate hybrid type. These individuals were allowed to interbreed freely and produced a numerous progeny, in which the larvæ were of four different types; white without spots, white with spots, yellow without spots, and yellow with spots. The second generation emerged from the middle to the end of July, 1905, and showed a huge preponderance of the *signaticollis* type. The census of a random sample taken the last week in July gave the following count:

<i>signaticollis</i> type.	mid type.	<i>undecimlineata</i> type.
1,244	1,192	367

These individuals were not removed from the colony; the census of the sample was made, the individuals put back, and the colony allowed to encounter the conditions and behavior which it would meet in a state of nature. Nine pairs, taken at random, of the *undecimlineata* type were bred out as pedigreed cultures during August and part of September, 1905, and gave uniformly an *undecimlineata* progeny. Seven pairs of the *signaticollis* type, which were bred out, gave uniformly a *signaticollis* progeny, and out of five other pairs there appeared individuals of the mid type and of the *undecimlineata* type, showing that some of the *signaticollis* type were hybrid in character. Six pairs of the mid type were also bred out as pedigreed stock, and showed themselves to be in every case hybrid.

The third generation was produced in August and early September, 1905. In this the larvæ were of the same four classes, but showed a huge preponderance of yellow larvæ (YIS). A count made late in August, when perhaps the bulk of the larvæ had entered into pupation, gave the following results:

Whs	WhS	YIS	YIs
205	227	849	321

The adults of Generation III. emerged early in September; a census made about the middle of September gave the following:

<i>signaticollis</i> type.	mid. type.	<i>undecimlineata</i> type.
2.452	827	218

showing again a marked decrease in the *undecimlineata* form, a lesser decrease in the intermediate hybrid type, and a much greater relative increase in the *signaticollis* type. These hibernated during the winter of 1905-1906 and emerged in June, 1906. They were allowed to interbreed freely. The population was not seen at the time of emergence, but in the fourth generation it was observed in July, 1906, and the *11-lineata* type and the mid type were nearly absent. The census made at this time, when the first generation of the year was apparently at its height, gave the following results:

<i>signaticollis</i> type.	mid type.	<i>undecimlineata</i> type.
3.275	45	7

These then inbred and the colony was next seen in September at about the middle of the month, when the census of the individuals in the colony was as follows in Generation V.:

<i>signaticollis</i> type.	mid type.	<i>undecimlineata</i> type.
1.823	6	0

These hibernated during the winter of 1906-07 and emerged in June, 1907, reproduced at once, and gave an abundant progeny which emerged as Generation VI. between the 10th and 25th of July. These when seriated gave the following results:

<i>signaticollis</i> type.	mid type.	<i>undecimlineata</i> type.
2.255	2	0

The second generation of 1907 emerged late in August and early in September, and of this generation the *undecimlineata* type was entirely absent, and the mid type practically so. These hibernated and when seen in the spring of 1908 only the *signaticollis* type emerged. Both generations of 1908 and both generations of 1909 have developed the presence of the *signaticollis* type only.

In 1908 individuals from this location were brought to Chicago and carried as pedigreed cultures in the laboratory. They have shown a complete gametic purity as far as could be determined, and none have been detected which were hybrid in character. In this colony, isolated in its location, through some process or other in hybridization or perhaps by selective factors, *signaticollis* has completely subjected and eliminated *undecimlineata*. Inasmuch as *L. undecimlineata*, when protected from crossing, lives well at Cuernavaca, and the selective action is very low, I am of the opinion that the swamping of *undecimlineata* is due to some process of hybridization. This opinion is fully justified by experiments conducted in cages which eliminate selective factors.

L. undecimlineata $\times$ *L. signaticollis*.

Exp. No. C. H. 47.4 Paraiso.

This experiment was begun in 1905, when one hundred individuals were taken from the standard colony of *L. signaticollis* at Cuernavaca, and, with an equal number of *L. undecimlineata*, from El Hule, were planted upon a vigorous growth of their food plants in a clearing made in the Foot Hill Rain Forest, in the Paraiso district not far from Ojos de Agua, in the Canton of Zongolica. They were observed to intercross freely, but there was a preponderance of *undecimlineata*-like forms, with a few intermediates, and only small numbers of the *signaticollis* type in the first hybrid generation. The census made of the first hybrid generation was as follows:

<i>signaticollis</i> type.	mid type.	<i>undecimlineata</i> type.
0	56	1,342

A third generation was produced in late November, and in that generation there were no *signaticollis* forms visible; there were only a few of the hybrid intermediate type and these all closely approximated the *undecimlineata* form. The census obtained late in November was

<i>signaticollis</i> type.	mid type.	<i>undecimlineata</i> type.
0	11	1,134

In 1906, 1907 and 1908 these cultures were allowed to shift for themselves, and the food plants were nearly swamped by immigration into the glade of plants from the surrounding Rain Forest; in fact, the whole culture was allowed to engage in a most desperate struggle for its existence. As far as the beetles were concerned, this was simply a struggle for food. In 1908-1909 the inroads which had been made by other plants had so reduced the number of *Solanums* that the food supply was inadequate. During these years, however, no trace of the *signaticollis* type has ever appeared. In 1908, material of the *undecimlineata* type was taken from this culture to Chicago, and there subjected to the tests of pedigree analysis, but without any trace of the *signaticollis* form appearing. In both experiments, however, at Præsidio and at Cuernavaca, the resulting materials were different in the gametic make-up from the original species. Superficially, these stocks could not be told from the natural species, but when used as the basis of experiment under control conditions, it was found that there resulted a difference in the behavior of the subsequent hybrid generations, clearly indicating a change in the gametic constitution of these groups of individuals.

Other experiments involving the same two species are in progress, under desert conditions at Tucson, Ariz.

Competition Hybridization Experiments Between L. decemlineata, L. oblongata and L. multitanziata.

A series of experiments, more conclusive and under better conditions, has been carried on, using three species: *L. decemlineata*, *L. oblongata* and *L. multitanziata*. Of these, in nature, *L. decemlineata* is limited solely to the United States and southern Canada; *L. multitanziata* entirely to the southern portion of the plateau of Mexico, and *L. oblongata* to the Balsas Valley and the Oaxaca-Guerrero Highlands. These species intercross freely under experimental conditions and present the following contrasting characters for consideration. The general ground color of the larvæ of *L. decemlineata* is wine red, that of *L. oblongata* and *L. multitanziata* chrome yellow. *L. decemlineata* and *L. multitanziata* have two rows of spots along the side in the larvæ,

while *L. oblongata* has one. *L. oblongata*, as shown in Fig. 4, is long and oval in outline; *L. decemlineata*, as shown in Fig. 4, is more rounded; and *L. multiteniata* is robust in type. There are also color differences between the species, which need not concern us here. Three experiments will serve to illustrate the purpose of this paper.

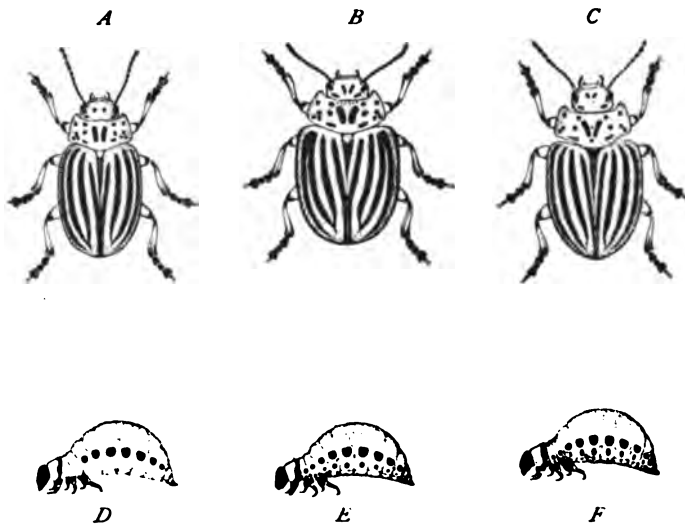


FIG. 4. Arranged to show some of the essential differences between the species: *L. oblongata*, *L. multiteniata* and *L. decemlineata*. (A) Showing the form and characteristic markings of the adult of *L. oblongata*. (B) Adult of *L. multiteniata*, showing the more robust form and somewhat different type of general color pattern, sharply distinguishing it from both of the other species. The elytral ground color is often dark ochre, sometimes even reddish. (C) The type of *L. decemlineata* used in these experiments, somewhat intermediate between the two other species in body form, and to a certain extent in markings. (D) Showing the side view of a full grown larva, with its color pattern. The ground color is yellow and that of the adult somewhat variable. (E) Adult larva of *L. multiteniata*, with the characteristic color pattern. Ground color is yellow as in *L. oblongata*, but darker. (F) Shows the characteristic color pattern of *L. decemlineata*; the ground color of the larvæ is wine red.

L. decimlineata × *L. oblongata* × *multiteniata*.

Exp. No. C. H. 156.2 Balsas.

In 1905, twenty *L. decemlineata*, from pedigreed culture Ex. No. 99, g. X from Chicago; twenty *L. oblongata*, from pedigreed culture at Cuernavaca, and twenty *L. multiteniata*, derived from

an isolated standard locality in the valley of Mexico south of Guadalupe, were placed on an isolated island in the Balsas River. This island was fairly well covered with a growth of *Solanum rostratum*, or a closely related form, upon which all three species would feed. As far as could be discovered, the island was devoid of any individuals of *L. oblongata* which occur very sparingly in that general region, and the neighboring banks of the river and the islands were all searched, but they afforded no trace of *L. oblongata*. These introduced beetles were allowed to breed and gave the first hybrid generation in August, 1905. In this generation only the adults were seen, and of the adults we could recognize definitely five forms:

(A) Those which on inspection appeared to be wholly *L. decemlineata*; (B) those which appeared to be wholly *L. oblongata*; and (C) those which appeared to be wholly *L. multitanziata*. There were individuals which were manifestly intermediate hybrids, in form, punctuation and coloration, between *L. decemlineata* and *L. oblongata* (D); and between *L. decemlineata* and *L. multitanziata* (E). Of these five forms a census was made with the following results:

A	B	C	D	E
327	371	142	1,439	246

All the individuals were allowed to remain in the colony and interbred freely in August, giving early in September a second generation, of which the following census was made:

A	B	C	D	E
46	101	90	1,292	210

These hibernated during the winter of 1905-1906, and were not seen again until September, 1906, in the fourth hybrid generation of the culture. At this time the dominant form was manifestly a combination between *L. decemlineata*, *L. oblongata* and *L. multitanziata*, with the *oblongata-decemlineata* attributes in excess of those of *L. multitanziata*. (A combination between classes D and E of F_1 and F_2 .)

A	B	C	D	+	E
7	25	12		2,210	

The huge preponderance of this complex type, which was neither one nor the other of the three species, suggests at once, of course, that the results could not be due to any selective process, because the type was not one of the original types but a hybrid complex which was preserved because of its fitness for the location in which it was living.

The wintering condition of 1906-1907 were especially rigorous, at least as judged by the number of beetles that I found in that location in 1906-07, when the following census was made:

A	B	C	D	+	E
0	0	4		422	

This shows that during the winter practically only the hybrid combination was able to survive. These reproduced and gave a progeny late in July, 1907. An inspection was made early in August when I found only the dominant type present in the fifth hybrid generation.

A	B	C	D	+	E
0	0	0		1,877	

The culture was not seen again until the spring of 1908, when a considerable number of the dominant form of the sixth hybrid generation was found emerging. These were taken to Chicago and subjected to analytical experiments and were found to breed true, both in group and in pedigreed cultures, with this exception, that in both group and pedigreed cultures, there occurred from time to time sporadic variants often standing a considerable distance apart from the rest of the population, which, when inbred, either with sports like themselves, or back to the parent type, gave behaviors which in every way are comparable to the behavior observed in many of the forms which are supposed to have arisen by a mutative process. These strains were kept through the years 1908 and 1909, and gave results which strongly suggested that the interpretation of a mutative period, as de-

scribed by De Vries in *Oenothera Lamarckiana*, may well be the variability which follows complex processes of hybridization.

L. decemlineata $\times$ *L. oblongata* $\times$ *L. multitanziata*.

Exp. No. C. H. 156.4 Escamela.

In 1906 operations were begun at Orizaba, and in May the same three species from the same original stocks were mated. Conditions at Orizaba are decidedly different from those in the Balsas Valley. The city is 2,000 feet higher in altitude and the climate is very different. In the Balsas Valley during the summer the days are bright and hot, with evening showers. At Orizaba, in the location chosen at the foot of the Sierra Escamela, it is never above 90° even on the hottest days, and the nights are always cool, owing to the downward draught of cool air from the mountains which flows over the valley at night. The relative humidity is high at all times, and the precipitation during the season was 74 inches.

Under these conditions the crosses which were made thrived as far as certain members were concerned; the *L. multitanziata* individuals were decidedly reduced by the conditions under which they were living and the *L. oblongata* individuals were hampered considerably, but to a lesser degree. Crossing was observed, however, among the component species in all directions, and progeny emerged in July, showing a combination to have been formed between *L. oblongata* and *L. decemlineata*, with the *L. multitanziata* type and attributes practically wanting. The population, when examined, showed individuals which were apparently dominated by *L. decemlineata* (A) to the exclusion (as far as visible) of all others; individuals which were very clearly intermediate between *L. decemlineata* and *L. oblongata* (B); and individuals which were more or less intermediate between *L. decemlineata* and *L. multitanziata* (C). Of these the intermediate between *L. decemlineata* and *L. oblongata* existed in by far the greatest numbers, as shown by the following proportion:

A	B	C
131	397	92

In as much as this experiment was conducted in a large cage and not in the open, it was manifestly impossible to utilize all the individuals which emerged, so a reduction was made for the matings for F_2 , excepting that any extreme or rare types were given every advantage over the more common types. The following materials were selected at random from the different groups as parents of the second generation:

A	B	C
3♂	3♂	3♂
3♀	3♀	3♀

These inbred rapidly during July and at the end of August gave a second generation which was uniformly an intermediate between *L. decemlineata* and *L. oblongata*.

A	B	C
0	589	0

This was especially true of the adult characters. The larval characters, however, were also variable and appeared to be less blended into a homogeneous group.

The culture hibernated from early September, 1906, to June, 1907. During this period a very great mortality occurred, which was due very largely, I think, to the fact that the culture would probably have reproduced a third time in 1906 if it had been supplied with food and proper conditions.

These individuals in 1907 reproduced and gave a pretty uniform progeny of the blended type between *L. decemlineata* and *L. oblongata*, generation III.

A	B	C
2	476	0

A fourth generation was obtained in late August and early September of the same year, which possessed the same attributes as the third generation. In nature, this culture was not carried beyond that stage, but material from the culture was brought to Chicago and carried through the winters of 1907 and 1908,

and the summer of 1908 and part of 1909. It was subjected to various analytical experiments, all of which tended to show that the type was a relatively stable one. Individual pairs, when inbred, gave a very definite pure line culture, and groups mated at random gave the same result; but, as in the colony in the Balsas River, there appeared sporadic individuals, widely separated from the parent stock, which, when inbred, behaved very much as mutants are supposed to do.

L. decemlineata × *L. oblongata* × *L. multitanziata*.

Exp. No. C. H. 156.8 Tucson.

A culture of the same material was placed at the Desert Botanical Laboratory of the Carnegie Institution in the desert of Southern Arizona at Tucson near the foot of Tumamoc Hill. In this experiment two males and two females of *L. decemlineata*, from Exp. No. 99, g. XIII, from typical stock at Chicago, two males and two females of *L. oblongata* from Exp. No. 619, g. XI, and two males and two females of *L. multitanziata*, from Exp. No. 543, G. XI, were mated in the early part of June. This culture was confined in a cage six feet square on the ground and three feet high, covered with wire eighteen meshes to the inch, thus eliminating all selection by insectivorous enemies. *Solanum rostratum* was supplied as food in sufficient quantity. During June and July these reproduced abundantly and gave a large progeny which emerged late in July and early in August. In this first hybrid generation at Tucson there was, as in the other cultures, a blending of the materials introduced into the experiment, but in this culture *L. decemlineata* was the dominant member of the cross, although not completely.

In the larvæ six types were observed:

1. Those which on inspection appeared to be *L. decemlineata*.
2. Those which were *L. oblongata*.
3. Those which were *L. multitanziata*.
4. Those which were intermediate between *L. decemlineata* and *L. multitanziata*.
5. Those which were intermediate between *L. decemlineata* and *L. oblongata*.
6. Those intermediate between *L. oblongata* and *L. multitanziata*.

It was, of course, impossible to tell on inspection what the constitution of each of these types was.

Five classes of adults were recognized:

- (a) Those which were clearly either pure, or dominants of the *L. oblongata* type.
- (b) Those which were clearly intermediate hybrids between *L. decemlineata* and *L. oblongata*.
- (c) An *L. decemlineata* type in which *L. decemlineata* was in the main dominant, but which exhibited a variable range of variability.
- (d) Intermediate hybrids between *L. decemlineata* and *L. multiteniata*.
- (e) Forms which were either *L. multiteniata* pure, or heterozygotes in which *L. multiteniata* was completely dominant.

Out of 1,857 adults seriated, the following census was made:

A	B	C	D	E
47	29	1,311	261	103

This census shows that while *L. decemlineata* is either the dominant or prepotent member of the combination, it did not come out of the mixture entirely without contamination.

This experiment was continued in a cage exactly like the first, and the following materials were taken at random from the first generation as the parents of Generation II.

A	B	C	D	E
2♂ 2♀	2♂ 2♀	6♂ 6♀	3♂ 3♀	3♂ 3♀

This material immediately began breeding and gave during the month of August a large progeny which emerged early in September, and immediately went into hibernation. When seriated, this material gave the following results:

A	B	C	D	E
0	29	247	42	0

These passed the winter of 1908-09 in the ground and emerged in June, 1909. All that emerged were allowed to reproduce in

the cage and were supplied with food as fast as it was consumed. These gave a very large progeny which appeared to be uniformly of the dominant types of the first and second generations. Seriation of the material obtained from Generation III. at the end of August, 1909, gave the following results:

A	B	C	D	E
0	5	362	8	0

I then mated at random for the parents of Generation IV., one male of B, the only one that could be found alive, three males and three females of C, two males and two females of D, and none of E, they being absent. This material bred at once and gave in the fourth generation a considerable progeny, which were all of the dominant type.

Material from Generation IV., brought to Chicago in August, 1909, placed in hibernation under experimental conditions and brought out to breed in the middle of the winter, has shown that the dominant type is a fixed type, and that it breeds true and does not split in subsequent generations. The only splitting is that which occurs in rare individuals in from two to three per cent. of the progeny, which stand apart from the general population as sports. These cases are practically the reappearance of one or the other of the component characters or combinations thereof that went into the cross, and they do not represent in this experiment anything in the way of characters new to the genus or family, as DeVries states to be true of his mutants; rather, they are simply the characters obtained from the different parents from which this complex has been built up.

The same combination of material was made in Chicago in 1908, and was run through essentially the same procedure as that of the Tucson experiment, with this difference in the result, that at Chicago *L. decemlineata* completely dominated the culture to the total exclusion, as far as analysis has been able to discover, of the presence of the other parents.

These experiments in synthesis represent what might happen in a state of nature when species which can hybridize migrate from one place to another and intercross. No one realizes

better than I the complexity of experiments of this kind, the difficulties involved in the analysis of the results, and the caution that should be exercised in making statements from them. It seems certain from these experiments, as far as they have been carried out, and they are by no means complete, that we may definitely conclude that when like materials are combined under different natural environments, differences in the products, depending upon the conditions under which the combination take place, result. It is certain that the type which came out of the culture in the Balsas Valley was quite different from that which resulted from the cultures at Orizaba, and these are different from the dominant type which arose at Tucson.

Whether or not the dominant types resulting in these experiments differ sufficiently to be called species is a matter of opinion. To judge by analogy, I suspect that if a systematist had found these materials in nature they would have been classed as species, or at least would have been given the value of a variety. Since their history is known, I presume that they lose all claim to any specific or varietal distinction, from a systematic standpoint. The experiments, however, clearly indicate that the process of hybridization carried out under divergent conditions in nature gives identical results, as far as principle is concerned, with the crosses carried out under divergent conditions in experiment. It is true that these cultures are group cultures and are synthetic and not analytic, but a series of experiments is being conducted under diverse natural environments, which gives promise of confirming the general point, that conditions incident or external to the germ cell at the time of fertilization may profoundly modify the behavior and the relationships of the characters entering into the crosses.

One point of very considerable interest is the behavior of these dominant types in exactly the way in which DeVries's *Oenothera Lamarckiana* behaves, giving in each generation a greater or less number of rather divergent individuals, which, when inbred, are found to be stable germinal variations. I shall report upon this more extensively in another paper.

Bateson (1902, p. 153) has suggested that the mutations observed by DeVries in *Oenothera Lamarckiana* are in reality due to some

sort of hybridization behavior. I am of the opinion that Bateson's suspicion is probably justified, at least in some instances. I have no experience with plants and especially none with *Oenothera Lamarckiana*, but my experience with these synthetic experiments has suggested that the type of behavior which DeVries has discovered, and upon which he has built an all-inclusive theory of evolution, is in reality nothing more than the reappearance from time to time of attributes brought into a strain by hybridization, and which reappear in every generation, or in frequent generations, by some process akin to Mendelian segregation.

It seems unreasonable to advance, as has DeVries, the idea of a pre-mutation period, with a gradual development of invisible pangenes, and then a final bursting of these pangenes into a full-fledged mutation period, followed by a gradual dying away of the mutation period which leaves a species in a condition in which it does not produce these sports. Rather, the explanation which Bateson suggested, and which I have shown to be capable of creation in these synthetic experiments, is far more plausible and more likely to be the real explanation of the type of behavior found.

This raises a very large question—one that has been raised many times—as to whether natural species may not be hybridization complexes rather than pure line cultures isolated by some sort of selection, as has been presupposed since the time of Darwin. I have found that in nature, crossing, especially between these chrysomelid beetles, is by no means uncommon, and very frequently results in adult progeny in nature, some of which have been described as species. These natural cases of hybridization have been observed in the last half dozen years along the edge of the Mexican plateau. Some other species of chrysomelids from the same general region, especially some species of *Labidomera*, have a variability strongly suggestive of a similar origin. I have found that *Labidomera suturella* Chev., of which many sharply marked variations have been described, gives a variability in pedigreed cultures that is strongly suggestive of the species having arisen through a process of hybridization. On the high volcanic plateau of Toluca there is another type

rather closely allied to *L. multiteniata*, which is also suggestive of having arisen, or of being in the process of arising, through hybridization.

These conditions in nature are of course difficult or impossible to check and verify, because the past is absolutely unknown, and little or no indication of what it has been can be obtained from any source. The materials in museums and the records by systematists are utterly useless for this purpose. Apparently, the only way of attacking this problem is the one which I have adopted of placing colonies in isolated locations, or in cages, there to carry out the process of interbreeding and forming of hybrid combinations as would occur in nature.

DISCUSSION.

Neo-Mendelism, The Factorial Hypothesis, and Theories of Germ-Plasm Composition.

The essence of Neo-Mendelism is based upon the actual experimental evidence of many workers, and is the idea that such of the attributes of organisms as show this type of behavior are the product of two factors which come into the fertilized egg, one from each parent, and that in gametogenesis these factors are distributed among the gametes by some process which is symbolized as "segregation." This factorial point of view is in no wise, of necessity, to be tied to or confounded with such speculations as the id-determinant-biophore fabric of Weismann, nor with the pangene complex of DeVries, which have no foundation in fact.

I doubt very much if Davenport (1910) will find many Neo-Mendelians willing to subscribe to his statement—"In studying heredity our attention must often be focused on the ontogenesis of the different characters, and we are sometimes inclined to regard the adult character as the product of the course of ontogenesis. But this is a superficial way of looking at things; the determiners of all characters are in the germ-plasm and together they direct the development of one part after another in orderly succession; a modernized form of the preformation doctrine seems logically necessary." That the determiners of all charac-

ters are in the germ-plasm is something we do not know, and to say that together they direct the development of one part after another in orderly succession puts upon these determiners a burden of great responsibility, almost of intelligence, and makes necessary some coordinating mechanism behind it all.

What we do know is that in some way, as yet unknown, in the germ-plasm there is conditioned the basis upon which the attributes of the future organism are to be built. It is a fact of experience that the germ cells give, on being combined at fertilization, results which suggest that the germ cells are of unlike potentiality or constitution with respect to a given character, and this is further strengthened by experiments wherein the results are exactly predicable when the germinal constitution is known. What this difference in the gametes is, we do not know, but observed behaviors are interpreted as being, most probably, due to the mechanical separation into different germ cells of whatever is it that produces the contrasting attributes—segregation during gametogenesis.

There is, furthermore, a very considerable amount of evidence, aside from that obtained by Neo-Mendelians, to substantiate this factorial point of view. The Mendelian behavior is most commonly found in color and color characters, and in color characters two definite general processes are involved; first, the production of the pigment to produce color; and second, the localization of this pigment in definite positions in the organism. The color itself is known very definitely to be, in a large number of cases, if not generally, the result of the interaction of two chemical substances. Riddle (1909) brings together from diverse sources the knowledge available concerning the formation of the melanin pigments, and, as far as known, the production of these pigments is uniformly due to the existence of an oxidizing agent—tyrosinase—and a substance which is oxidizable—tyrosin or allied compounds. In the test-tube of the physiological chemist, the tyrosin will remain tyrosin indefinitely unless it be oxidized by the tyrosinase into some simpler substances, when the color-forming compound appears, and exactly the same is true in the living organism. Also the tyrosinase will indefinitely remain tyrosinase, without the formation of color, unless

tyrosin be present. There is in this production of melanin pigment exactly the requirements of the Neo-Mendelian factorial hypothesis, as suggested by Cuènot (1903) and developed by many workers in the last few years—two things which must be brought together to produce a definite end result, or if one be absent then the one present is incapable of producing the customary result, *i. e.*, pigment.

In plants, it has been shown by Correns, by Bateson and his students, and others, that two factors are necessary to produce color; and in *Antirrhinum*, Wheldale (1907) and Wheldale, Maryat and Sollar (1909) have a considerable body of evidence to show that there are specific chemical substances—chromogens—to be oxidized to produce the colors of the flowers. Bateson (1909) has clearly stated that this does not mean that any substance, as a specific substance, or entity, is segregated in the germ cells; it simply means that a capacity for the production of either one or the other, or both of them, must be present in order to produce definite results. This capacity, as far as my experience goes, seems in all cases to be a property of the whole organism, which during ontogeny, manifests itself epigenetically, now in one part or character, now in another, and a very simple change at the start may conceivably result in extensive end results. I am, while fully convinced of the truth of the behavior of characters Mendelianwise, equally unconvinced, as the result of ample experience, that the process is in any way due to a particulate composition of the germ-plasm, or to any sort of preformation, and as far as I am aware, no Neo-Mendelian has thus far attempted to form any conception of what it is in the germ cells that is productive of the observed results, and only rarely do we find such concrete statements of preformation as that made by Davenport.

Castle (1909, page 68) expresses more accurately the current Neo-Mendelian conception of these factors: "In what form, it may be asked, are we to suppose that the various assumed factors exist. Do they occur as so many different substances lying side by side but unmixed in every reproductive cell? . . .

"It is, we think, not necessary to suppose that there exist in the minute germ-cell as many complex organic substances as

there are activities of the cell; neither is it necessary to suppose a different substance present for every independent factor identified. The various independent factors may have a basis no more complicated than that of so many atoms attached to a complex molecular structure. Experiment shows that the factors may be detached one by one from the organic complex. The discontinuity of their coming and going is entirely in harmony with the conception of them as components merely of complex molecular bodies."

If I understand Castle correctly, the atoms or groups thereof that are detached, are not of themselves the repository of capacities such that anywhere they would produce a given result, but rather, because of their absence in the complex group, the development of a character fails, and their presence is necessary to the appearance of the character in question. This conception is fundamentally different from the representative particle ideas of DeVries or Weismann, although the two ideas are not infrequently confused.

In the introduction (p. 1) I pointed out that the fundamental question which must be answered before we can get any real explanation of Mendelian behavior is, whether these "unit characters," are lesser entities conditioned by representative particles capable of mosaic rearrangement in the organism. It was further pointed out that to conceive of an organism as an entity analogous to a crystal, or as a mosaic more or less analogous in certain respects to granite or to orthoclase-feldspar crystals, expresses figuratively some of our experiences concerning the nature and composition of organisms.

There is, unquestionably, in all organic forms that which has the stability of form characteristic of crystals, and there is that which can be removed or replaced without in any way changing the basic form, exactly as impurities can be removed from or substituted for in crystals. Likewise, there are alternative conditions of existence of this basic form, which, like allotropic crystals, can exist only as one or the other form.

In germ cells the colloidal ground substance, which Lillie (1906, 1909) finds is not disturbed in its polarity and in its future developmental processes by powerful centrifugal force, may very

probably represent the more crystalline-like germinal basis, and any of the included granular substances or chromosomes which are capable of more or less alternation in relation and position, may conceivably in some way be connected with and indicative of the more superficial, removable, or changeable attributes.

The evidence derived from studies of accessory chromosomes and their segregation into germ cells during maturation, giving germs of different chromosomal constitution, is indicative of some difference in germinal composition. Whether these behaviors of chromosomes are the consequence of more fundamental germinal differences, or the initiation of subsequent germinal differences, is a problem for the future to decide. It does not of necessity follow that the chromosome is particulate, or the determiner of anything; it is at present an indicator of germinal difference which is coupled with whatever it is that determines the alternative nature of sex. If differences of this sort exist in one alternative character, there is no *à priori* reason why there may not be similar differences in others.

Our present experience indicates a germ plasm composition analogous to that of an orthoclase crystal, with a basis to which are added to or subtracted from through the reproductive process, attributes which become parts and properties of the whole. That the germ-plasm is such immortal material, irreversible in its action, that it possesses a oneness of constitution and ultimate destiny, is a conception based upon *à priori* dogmatic metaphysical concepts, and is a negation of the evidence of our senses.

Many years of experience with the problems of variation, heredity, and evolution, wherein germ-plasm constitution must play a vital part, have forced me to the formation of a conception of germinal substance like that expressed above. It is distinctly neither one nor the other of the older conceptions, but rather a mid-position, recognizing the elements of truth in both of the older conceptions, and is in entire harmony with the facts of experience. A presentation of my data and more extended elaboration of this conception cannot be attempted in this preliminary paper.

There is in this conception no element not in harmony with the existence of Mendelian behavior of characters, nor incom-

patible with a behavior in gametogenesis that would result in mechanical separation into unlike masses of germinal substance. No idea is expressed or implied in this conception of the nature of the physical state which conditions anything in the germ plasm, nor must the fact be lost sight of that this is a proximate conception of germinal substance and not an ultimate. At present in biology we have no business with ultimate conceptions, and the two thus far attempted of germinal constitution—the “particulate conception” and the “crystalline entity” are both equally dismal failures and equally useless as working hypotheses.

The conception herein set forward recognizes the following facts as regards organic constitution:

1. That there is in organisms a form basis, relatively unalterable as regards symmetry, pattern and arrangement of parts.

8. That there are in organisms an array of attributes capable of variation, but blending in heredity, forming blends and intermediates.

3. That there are in organisms an array of attributes which can exist only in a definite state of stability—they are either there or not there.

4. That there are in organisms characters that by crossing can be replaced by other more or less similar but different characters.

These four classes of attributes in some manner are conditioned by physical forces in the germ-plasm, and are, as far as we can perceive, the product of the past interactions between the germinal substances of past generations, and between these substances and the conditions of their existence and activity. Two chief series of physical events are at work; the series of events characteristic of any germinal substance—the product of its past history, the genetic forces—and the interaction of this substance with new germinal substances under the dynamic action of surrounding or incident forces. It follows from this that any germ cell is an epigenetic product of the two series of events necessary to the production of fertilized eggs and the resultant soma-germ complex, and the subsequent germinal changes may be large or small, in one or many of an unknown number of possible directions of modification; and while consequent somatic or germinal attributes may to us appear as either continuous or discon-

tinuous in results, in the interaction between the genetic state and the dynamic forces only continuity of process is probable on the basis of present physical knowledge.

This genetico-dynamic-hypothesis of germ-plasm constitution and modifiability, forms a convenient basis of departure for a host of evolution problems, and it is from such a theoretical basis that I have sought to study certain phases of the Mendelian phenomena.

Dominance.

The subject of dominance and recessiveness is at present decidedly an open question. What do we mean by dominance, and what is it that determines dominance? In the simpler cases, as illustrated by Mendel's peas and many others, there is no difficulty in stating positively which one of the two characters entering into a cross is the dominant member. Such examples are fairly abundant; they behave with considerable uniformity and represent a type of behavior for which there is some definite cause. What is it that makes one character recessive to the other? The Neo-Mendelian explanation is that one character is recessive and the other dominant because the recessive character is the absence of that which goes to make the dominant character. This explanation, however, while it may fit some characters, melanin pigment, for example, could not by any means fit all cases.

Thus, for example, in the experiments given in this paper, in crossing black stripes and no stripes, we may well conceive of the black stripes as being due to the presence of the two factors which produce black, and of no stripes as being due to the fact that one or both of the producing factors is absent. As far as the causation of color is concerned, this is actually the case, because I showed in 1903 that the black and brown color in these beetles is due to a substance in the cuticula which must be oxidized by an enzyme to produce the dark pigments. This is a clear example of the dominance of the presence over the absence, in some of the experiments and not in others. But the case is different in such crosses from that between tall and short stature in pea plants, tailed and rumpless condition in poultry, etc.; these must in one way or another be due to some sort of activity within the

organism. According to the theory of inhibitors, angora forms have long hair because the inhibitor of hair growth fails to act in one case, giving long hair, and acts in another, giving short hair; and poultry have tails because the inhibitor of tails fails to act in most cases, but acts rarely to give tailless birds, that is, the tail in birds is recessive to no tail, etc. This seems an extreme and unnecessary complication of the present hypothesis.

Unquestionably, something determines dominance in color, in tall and short peas, etc. Probably the most extreme cases of dominance are those which breed true without segregation in F_1 and subsequent generations. A behavior of this type is given in Exp. No. H 410, where the F_1 hybrid was exactly like the female parent and continued to breed true during the succeeding generations.

In Exp. No. H 409/411, there again appeared a dominant type, dominant to the complete exclusion of the other parent, which continued to breed true generation after generation; yet from the same parents there arose individuals which gave a different behavior, and both behaviors were based as far as any evidence is available, upon one and the same kind of germ cells. These cases represent dominance of the extreme kind, in which there is a total disappearance of the characters of one of the parents in the subsequent progeny. From these, we pass over such cases as those observed by Mendel and many others, to these F_1 hybrids in which the individuals are a blend and intermediate between the two parents. Such are given in several of the experiments cited in the body of this paper, and in some there were a series of gradations from the extreme of total dominance to the other extreme of an intermediate or blending condition between the two attributes.

In these blends, which are typical heterozygotes, the dominant attribute is diminished in manifestation in F_1 but appears undiminished in F_2 —but what shall we say of Exp. No. H 701 B, where in F_1 there appeared the *undecimlineata* type, mid type, and *signaticollis* type? In this series, from the same parents, the presence dominates the absence, blends therewith, is recessive thereto, in F_1 and only the mid types are heterozygous—all from one and the same pair of parents in each repetition of the experiment.

The question is, to what is this behavior and its variability due? The older writers confused dominance with prepotence, and the behavior was attributed to "strength," to the age of the parents at the time they reproduced, etc. As far as I can discover, the evidence for strength or age is anecdotal or very questionable in character, and in a long series of experiments I have been unable to get any evidence that age of the gametes or parents signifies anything in hybrid behaviors. Whatever is meant by strength no one can say, and to attribute behavior which gives dominance and recessiveness to differences in strength is meaningless. If by this "strength of germs" is meant energy for growth and development, we must, in order to utilize any such conception, know how much potential energy is stored in each germ—what the kinetic output would be when two germs were combined, and the increase or decrease of the kinetic output by conditions surrounding or incident upon the energy of the zygote. In other words, the friction of the developmental processes will vary and be as productive of resultant differences as are initial differences in the endowment of potential germinal energy, if such there be. Much more knowledge of germ-plasm physiology and dynamics is necessary before germinal energy can play any part in the explanation of hereditary behavior.

It has not thus far been shown that staleness of the germ cells is productive of any of the results which have been described. We know that germ cells become stale and that fertilization becomes difficult or impossible, and since development is slow or incomplete from stale germs it is possible that when germ cells that are stale are combined with those that are not, one or the other may dominate in the cross. I have attempted many experiments along this line, but thus far without any concrete evidence that staleness in itself is productive of any result.

In the present series of experiments there were involved in the elytral stripes of one parent:

1. Capacity to produce a substance capable of being oxidized to form colored compounds.
2. Capacity to produce an enzyme capable of oxidizing this substance.

In the other parent:

3. No capacity to produce either one or the other of the above.

The chief variables in these experiments were the conditions surrounding and incident upon the germ cells at the time of fertilization. All of the crosses herein recorded were made between individuals of the same age and always between vigorous individuals of their respective population; that is, the materials for crossing were always homogeneous as far as could possibly be determined, and I never mated an old male with a young female, or vice versa.

In these experiments I have succeeded in creating a series of behaviors in which the same characters are dominant to the complete exclusion of the others; dominant to a lesser degree, or in which there is a complete blend between the two in the F_1 generation, or the appearance of both parental types in F_1 and both breed true. As far as it has been possible to determine, the only variable in these experiments is that stated, and I am led to the conclusion that the conditions surrounding and incident upon the germ cell at the time of fertilization may be to a very considerable extent responsible for the determination of the dominant character in the cross and largely responsible for variability of such characters.

In Exp. No. H 701 B the behavior, where the recessive appears as a pure breeding race in F_1 , while by no means unique, is difficult of explanation on the factor hypothesis as at present understood.

It could not for a moment be maintained that external conditions are alone responsible for the determination of dominance or recessiveness, because it would be about as far from the truth as it is possible for one to get. External conditions have the same role in organisms that they have in any physico-chemical process, of accelerating, retarding, or changing the direction of the activity or of the reaction which is going on. The fundamental reaction in any of these hybrid crosses is that which goes on between the two physico-chemical complexes comprised in the combining germ cells. What that action actually is, is at the present time absolutely unknown. That it is due to combinations of the pangenes or biophores is a preposterous idea, and that a given ferment, a chromogen, of itself, or represented by any specific substance, is present as an entity in the germ cell is

likewise very doubtful. All that we are entitled to declare or believe on the basis of present actual evidence is that some condition in the physico-chemical constitution of these germ cells is present, that makes possible the appearance of one or the other of the two necessary factors which in many instances must be present in later ontogenetic stages, in order to produce a given result.

Relation of External Conditions to Dominance.

The question of dominance, while a vital one, has been somewhat wrongly attacked. I would hardly wish at the present time to attempt to account for the highly variable results that have been found in the dominance and recessiveness of characters by such explanations as germ contamination, variable potency, alternative dominance, or different types of latency, etc. It may well be true that there is variable dominance, but to what is this dominance due? It seems that many of the experiments in which variable dominance has been found and described were uncritical, and were carried on under the uncontrolled conditions of most breeding operations. In regard to variable dominance or alternative dominance, as a function of the gametic constitution of organisms, it is necessary that the operations should be carried on in such a way that surrounding or incident conditions are eliminated to the fullest extent; and experiments must be based, not upon one series, but upon parallel series of similar cultures. As far as Shull's (1908) attempt to explain this condition by various types of latency is concerned, there again the relatively gross conditions under which we are obliged to carry out most of our experiments leaves one open to criticism as to what the results observed were actually due. In other words, the question of dominance, as Bateson (1902) has suspected, is not entirely one of gametic constitution, nor is it one of external conditions, but it is a combination of the two, and this result seems to be fully borne out by the experiments cited in this paper.

Concerning the nature of the products which result from a given cross, much depends upon how dominance works and what it is that is present in the germ cells. The products

may well be modified and lead to endless confusion by this very fact which I have established, of the determining and influencing of dominance by external conditions. Thus, for example, in Exp. No. H 410, external conditions determined the whole future history of that culture. By the conditions surrounding the germ cells at the initial cross the total character of the race was determined for as many generations as I cared to continue the experiment. If this is generally true, and I see no reason why it may not be, then the determination of dominance, which determines also the resulting products, is a most vital factor in evolution.

Again, the variability in products which one finds, as, for example, the differences which MacDougal (1905) found between crosses of *Oenotheras* made by him in New York and those made by DeVries in Holland, when they were using, as far as could be determined, identical material, has a very direct bearing upon this point. MacDougal says: ". . . , the very differences between the results of the hybridizations, as carried out in Amsterdam and New York, suggest that the manner in which the various qualities in the two parents are grouped in the progeny might be capable of a wide range of variation. Many indications lead to the suggestion that the dominancy and prevalence, latency and recessivity of any character may be more or less influenced by the conditions attendant upon the hybridization; the operative factors might include individual qualities as well as external conditions."

This at once suggests differences in results, and difficulties that will arise through the carrying out of like experiments under unlike conditions. This phase of the situation is set forth to some extent in the second part of this paper in the experiments in synthesis. These experiments, while not susceptible of analysis along certain lines, indicate very clearly that the operation of the principles of alternative inheritance will be productive of diversity of results under diverse conditions when using homogeneous materials. This gives a clue which may be of paramount importance in the further investigation of the problem of the origin of species in nature.

In many organisms there exists a physiological isolation, which,

although the organisms may be close together, living in the same location, etc., by definite limitations in their reproductive activities prevents them from intercrossing. This may be due to incompatibility between germ cells, but quite often it is due to difficulties incident to copulation or to the penetration of sperms; that is, the difficulties are purely mechanical. Thus it might well rarely happen that an individual would arise of a character such that a cross could result; and if such a cross were made, a race having the attributes of one organism with the capacity for physiological isolation of the other, might easily arise and keep it from any further chance of intercrossing with other species.

CONCLUSION.

The experiments and observations herein given warrant the general statement that conditions external to a cross are important factors in determining the results thereof. This conclusion has been worked out in both normal and hybrid crosses, in crosses between races which have been created selectively, and between forms which arose as sports; and the second series of experiments in synthesis is sufficient warrant for attributing to this factor a considerable importance in evolution.

Underlying these, there are, of course, deeper factors than those with which we are dealing. The characters which behave Mendelianwise are in the main superficial, unimportant attributes of the organism, and only rarely are they the characters which would make for success or failure in the struggle for existence; they are most often color and specific characters, which, while fixed and vigorous in their behavior, are not important in the economy of the organism. These behaviors, beyond any question as to how and why, suggest the operation of something which gives a result best described at present in factorial terms; and that there are such things as later ontogenetic factors seems highly probable in many cases and absolutely certain as regards many colors. The knowledge that we have concerning melanogenesis leaves us no alternative in this respect.

Back of all this, however, is the fundamental question of how these germ cells, how this living substance is constituted, and what

is the relation in this complex of that which makes for the elaboration of tyrosin and tyrosinase in melanogenesis. What is it in this complex that localizes in a definite area the appearance of a pigment? What is it that combines into a definite pattern a series of attributes, some of which can be shifted and rearranged in the processes of hybridization? The problem of the constitution of the gametes of that which makes for form, for localization, for pattern, etc., is the fundamental problem; and as long as we fail to see clearly what the constitution of living matter is, such phenomena as these which we have been discussing must remain more or less superficial in our knowledge of the living organism.

There is nothing in the behavior of these attributes, in our ability to shift them and make new combinations, which, of necessity, commits one to any of the doctrines of preformation in pangenes or biophores, or to oneness of constitution and orthogenetic destiny. The situation, as regards alternative behaviors, should be free from the bias of biological orthodoxies, and to regard the organisms with which we are dealing as so many complex physical substances whose composition we are investigating, and among which we are seeking to determine the limits and laws of combination, will give the most rapid progress towards the end of a better understanding of the larger problems of the evolution of living substance.

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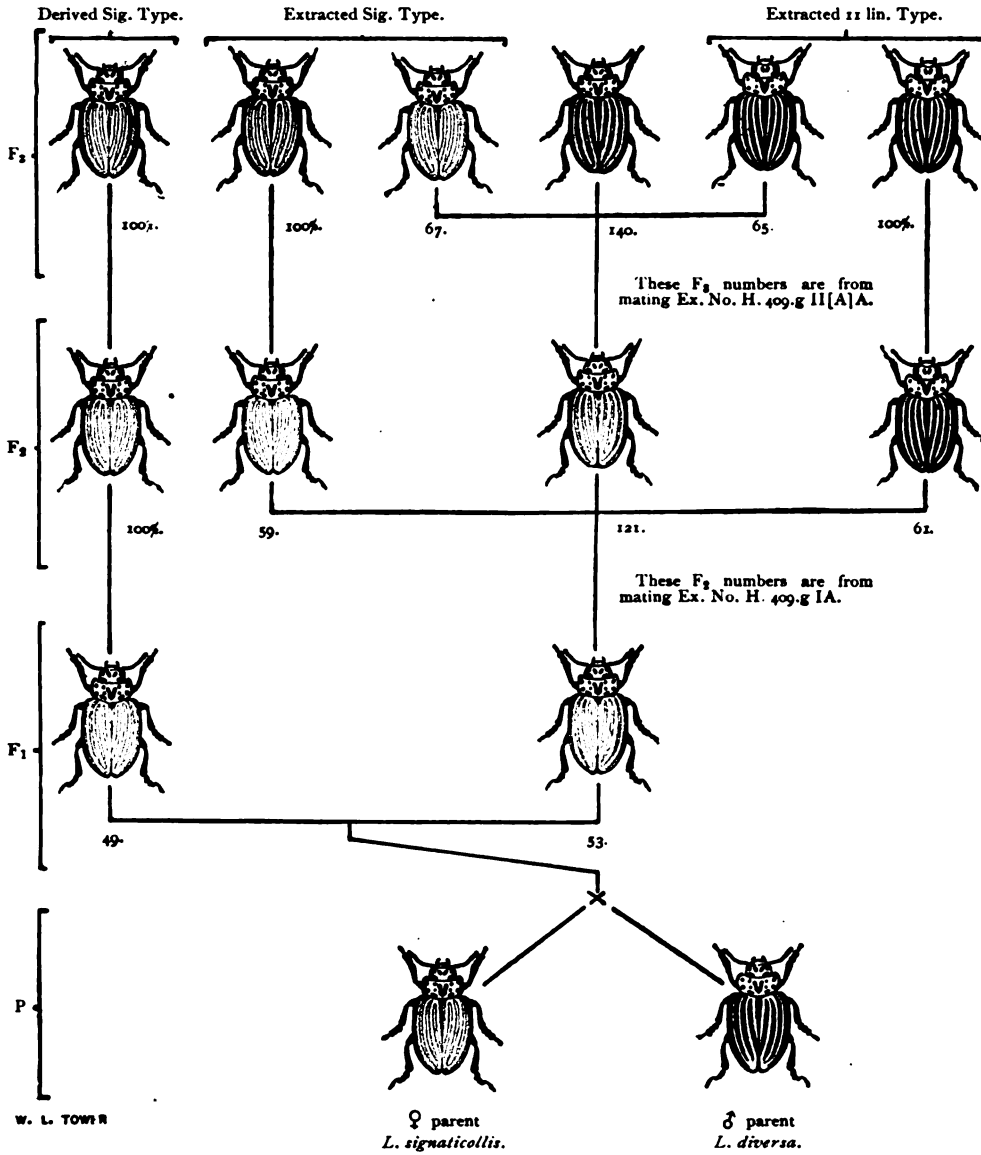
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EXPLANATION OF PLATE I.

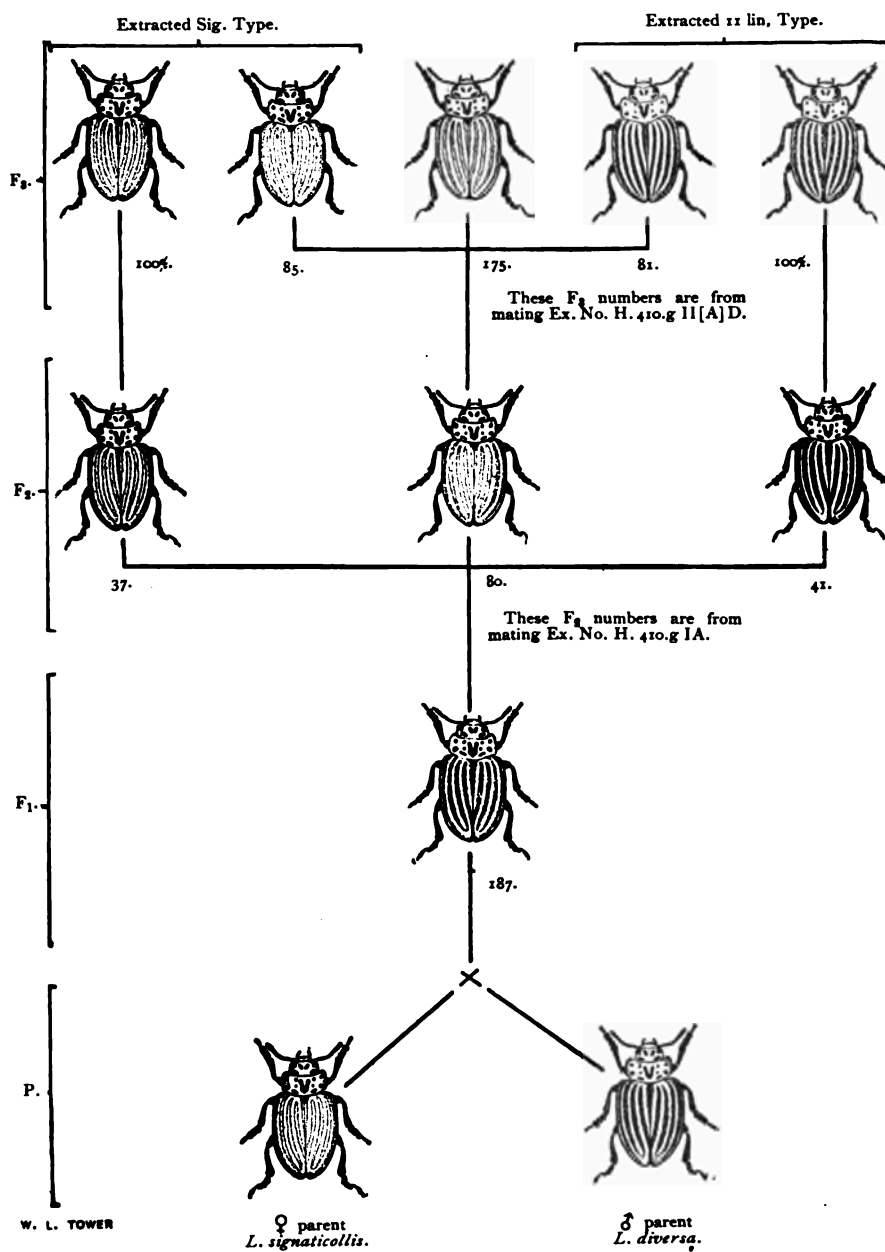
Note.—On all of the plates the pattern of the head and pronotum are not drawn with the true pattern and behavior which these cultures showed. The pattern shown is a basal one common to all the species.

Arranged to show the results obtained in crossing *L. signaticollis* ♀ × ♂ *L. diversa* under the conditions of Exp. No. H 409.



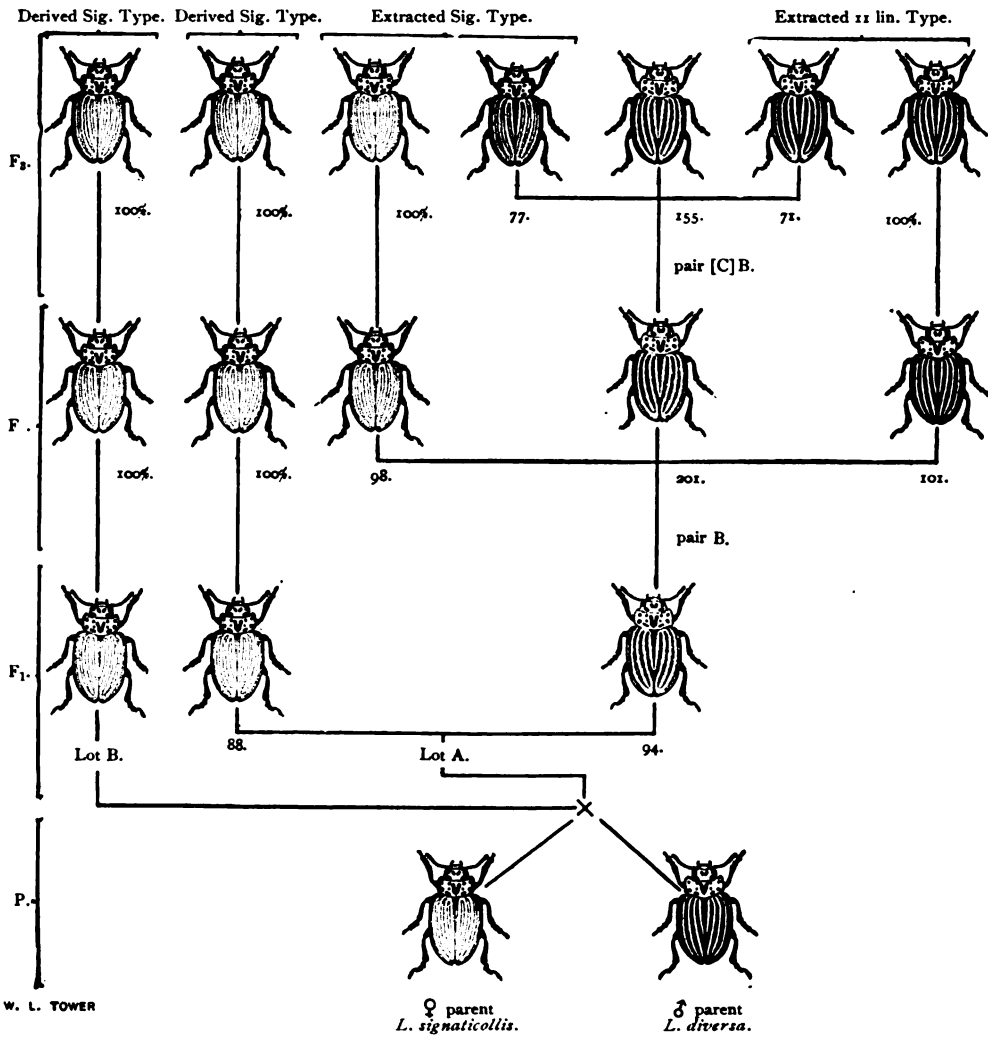
EXPLANATION OF PLATE II.

Arranged to show the results obtained in crossing *L. signaticollis* ♀ × ♂ *L. diversa* under the conditions of Exp. No. H 410.



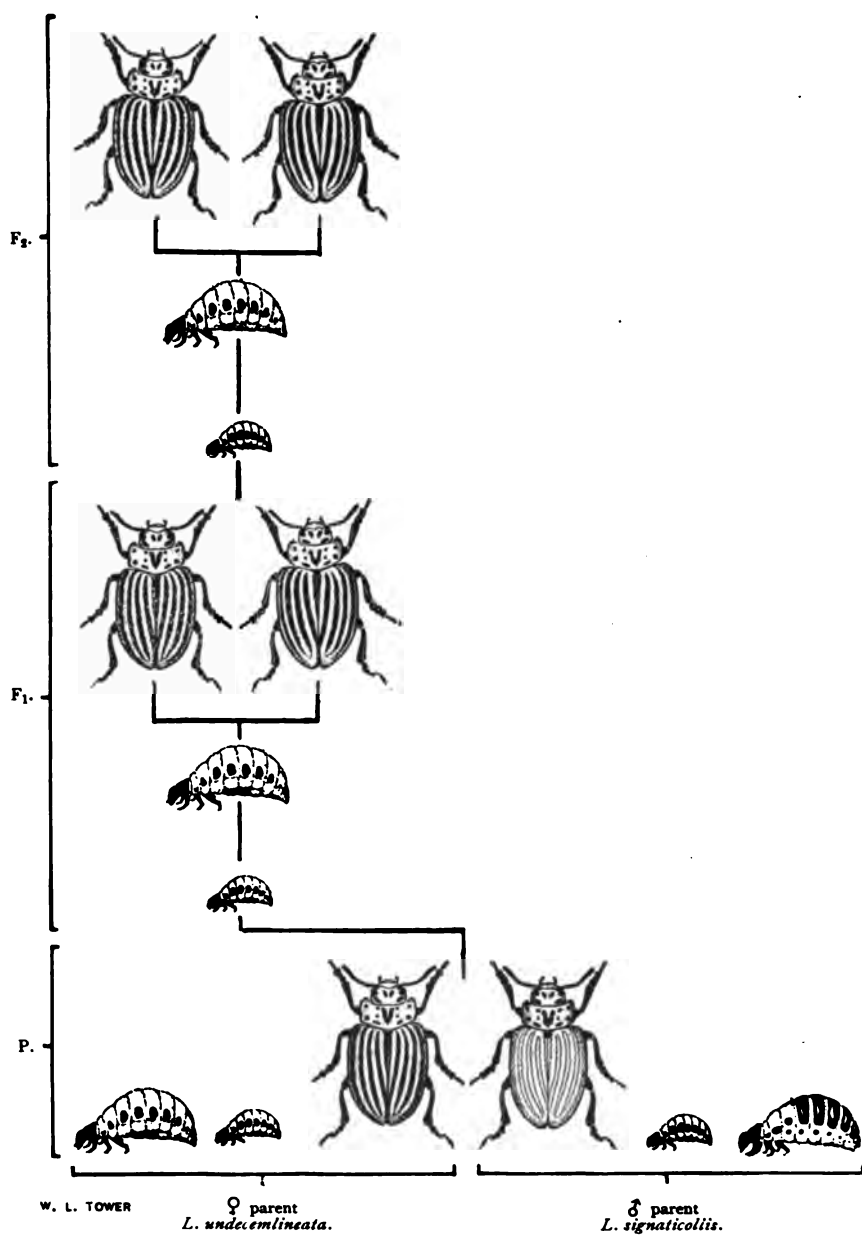
EXPLANATION OF PLATE III.

Arranged to show the results obtained in crossing *L. signaticollis* ♀ × ♂ *L. diversa* under the conditions of Exp. No. H 409 411.



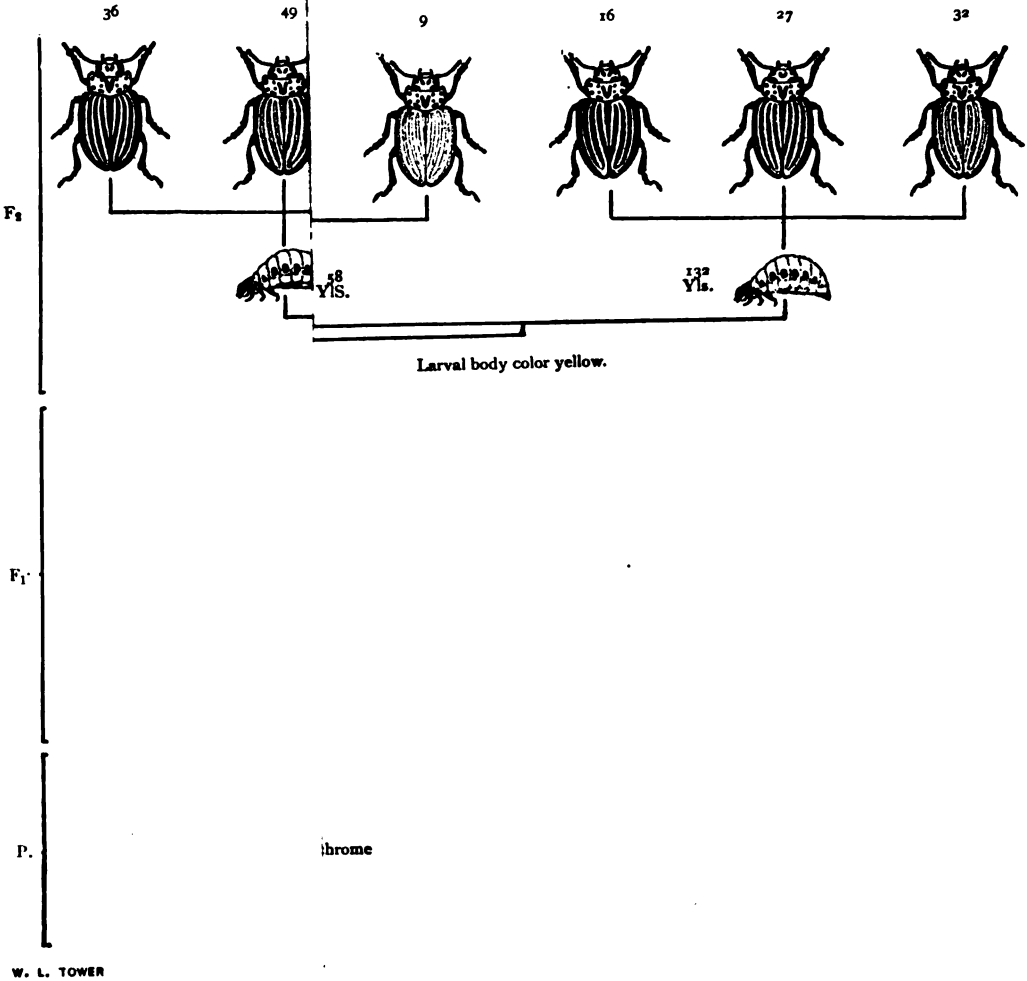
EXPLANATION OF PLATE IV.

Arranged to show the results obtained in crossing *L. undecimlineata* ♀ × ♂
L. signaticollis under the conditions of Exp. No. H 700 A.



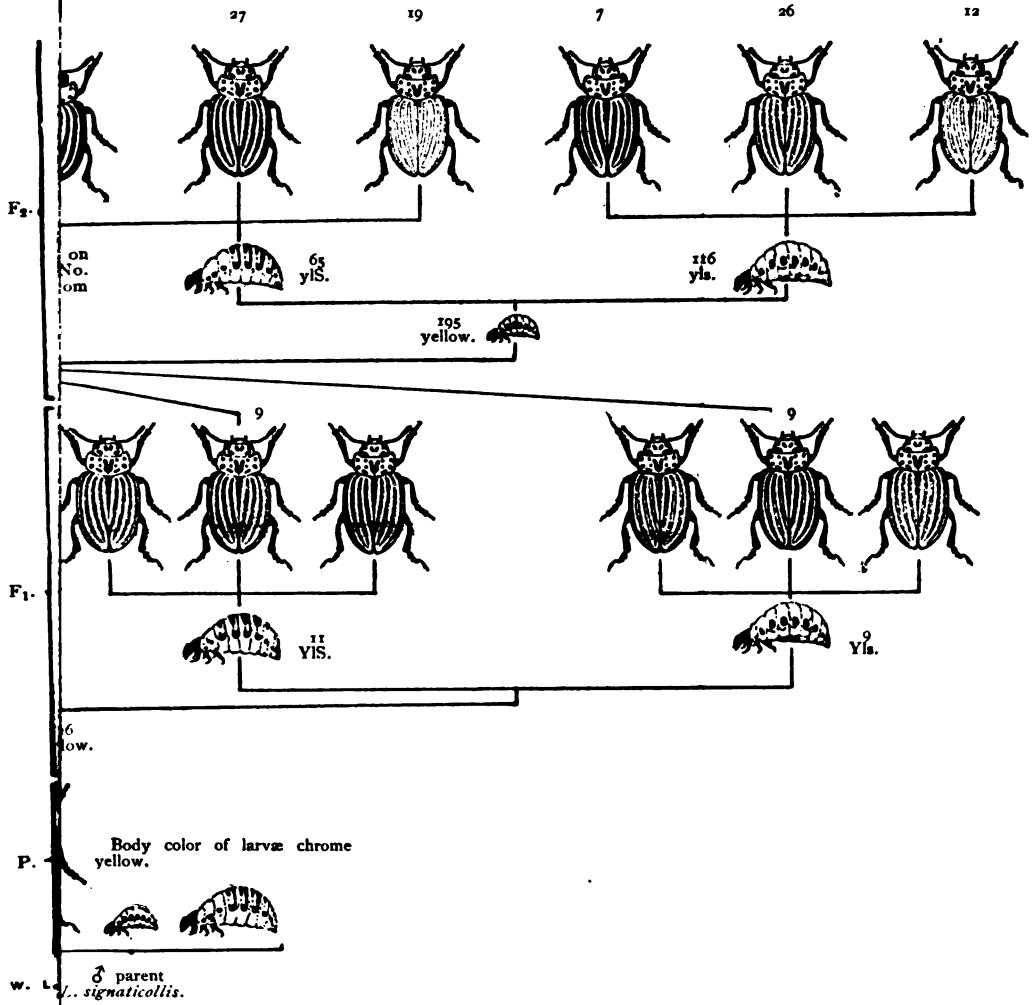
EXPLANATION OF PLATE V.

Arranged to show the results obtained in crossing *L. undecimlineata* ♀ × ♂
L. signaticollis under the conditions of Exp. No. H 700 B.



EXPLANATION OF PLATE VI.

Arranged to show the results obtained in crossing *L. undecimlineata* ♀ × ♂
L. signaticollis under the conditions of Exp. No. H 701.

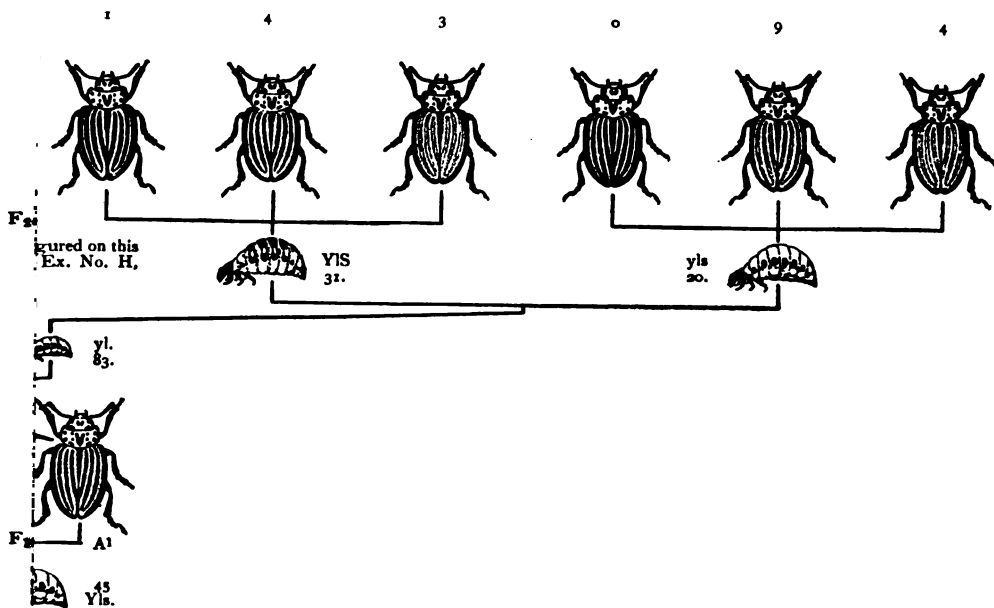


EXPLANATION OF PLATE VII.

Arranged to show the results obtained in crossing *L. undecimlineata* ♀ × ♂
L. signaticollis, under the conditions of Exp. No. H 700.

216

PLATE VII.



color of larvæ chrome

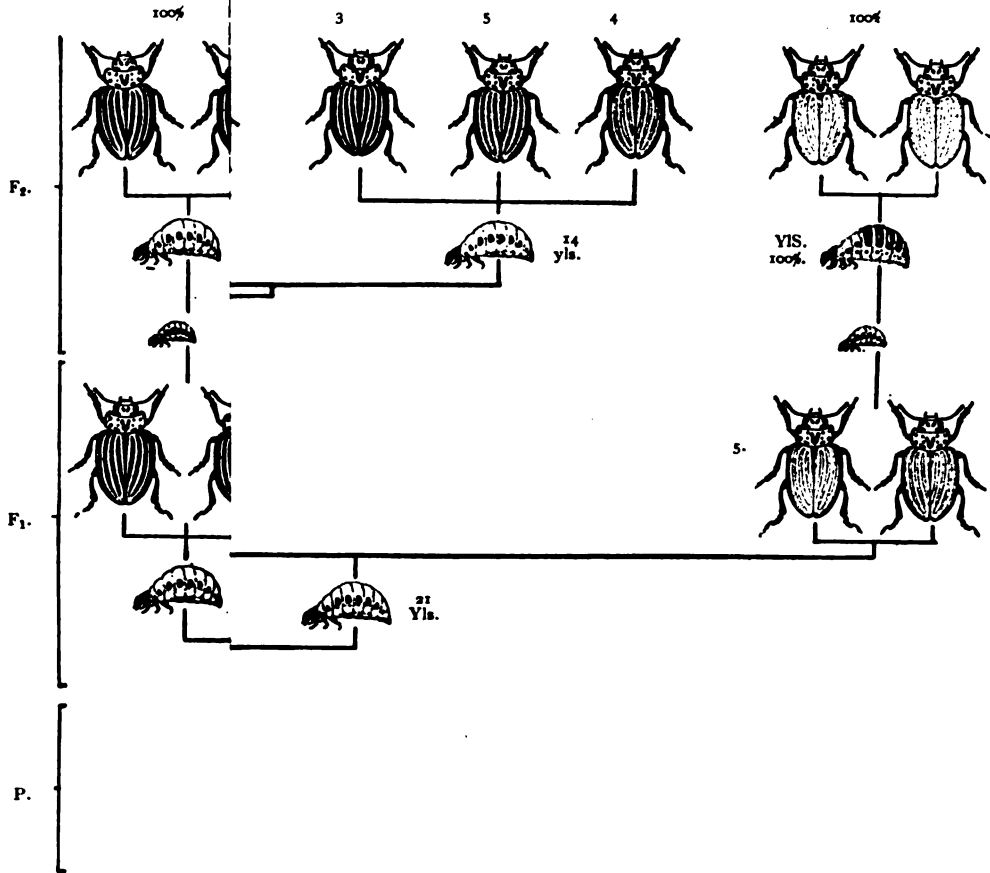
F



w. tent
collis.

EXPLANATION OF PLATE VIII.

Arranged to show the results obtained in crossing *L. undecimlineata* ♀ × ♂
L. signaticollis, under the conditions of Exp. No. H 701 B.



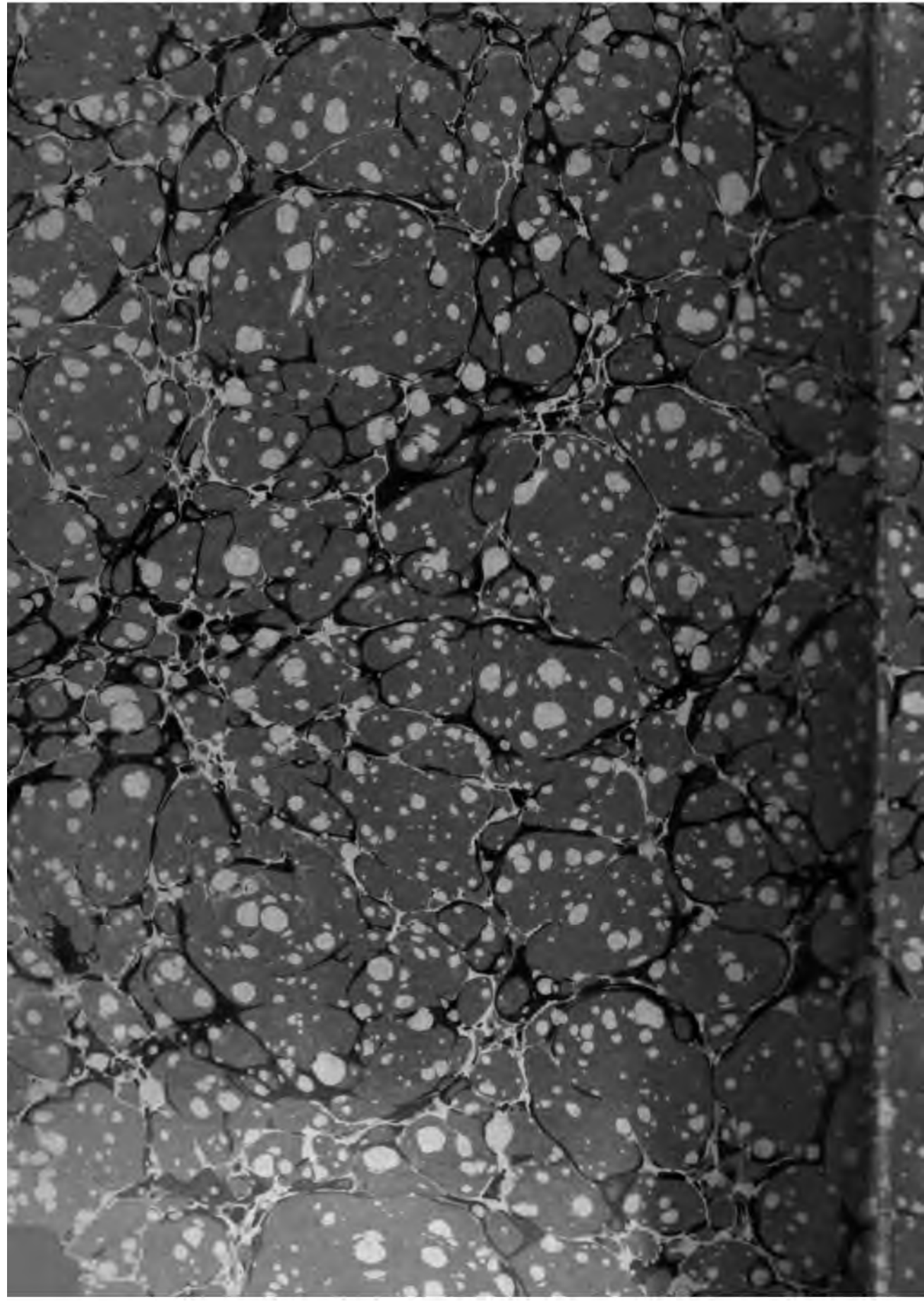
570.5

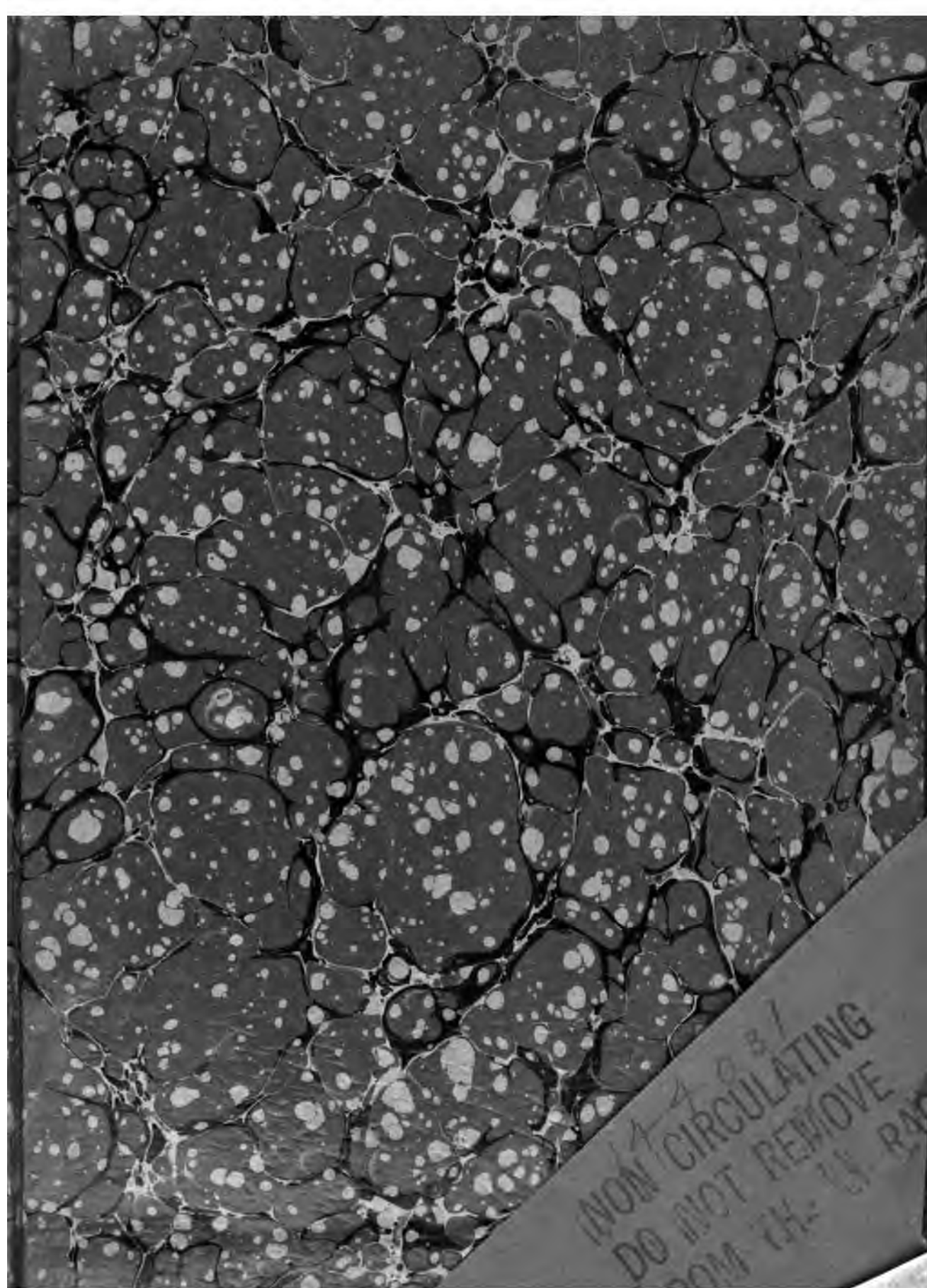
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DEC 1909

MAY 1910





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